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Carbohydrate Chemistry

VOLUME 8

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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 8

A Review of the Literature Published during 1974

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Preface

This Report, the eighth in the series, covers the literature available to us between mid-January 1974 and mid-January 1975. Strenuous efforts have been made to limit the size of this Report, in order to combat the rise in price of successive Reports in this series, but without prejudice to the type of literature coverage normally achieved.

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

Dr. N. R. Williams has joined our team of Reporters for Part I. We thank Drs. L. C. N. Tucker and F. Hunedy for reading and commenting on the whole of Part I, and Miss Moira Endersby for typing considerable proportions of this Report.

Once again 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.

J. S. B.

July 1975

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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
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

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

By

J. S. Brimacombe
R. J. Ferrier
N. A. Hughes
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.

It has been a particularly active year in monosaccharide chemistry, judging from the increase in the number of papers abstracted, with interest fairly evenly divided between synthetic and stereochemical aspects of the subject. The search for a protecting group that, like the trityl group, can be removed as a stable cation in glycosylation reactions but that can also etherify secondary hydroxy-groups has culminated in the use of the 2,3-diphenyl-2-cyclopropen-1-yl group (Chapter 4). Reference is made in Chapters 4 and 6 to a kinetic approach for calculating all ratios of rate constants characterizing the reaction of a diol (*e.g.* methyl 3-acetamido-3,6-dideoxy-α-D-glucopyranoside) with an alkylating or an acylating reagent; the information obtained helps to define the factors influencing the reactivity of a particular hydroxy-group and the relations between these factors, and avoids the misleading results that are sometimes given by product analysis alone.

From a synthetic viewpoint, it is pertinent to note that sulphonyloxy-groups at C-2 of β-D-glycopyranosides can be displaced with nucleophiles (Chapter 6). Syntheses of a number of interesting branched-chain sugars (*e.g.* aldgarose, vinelose, and 3-*C*-hydroxymethyl-D-riburonic acid) (Chapter 15) and of dihydrostreptomycin (Chapter 20) have been reported. The elegant chemical and physical studies used in elucidating the structure of sisomicin, a novel aminoglycoside antibiotic, make rewarding reading. The torrent of publications on nucleosides (Chapter 21) remained unabated in a year that has seen syntheses of the first 1′,2′-unsaturated purine and pyrimidine nucleosides.

Recent progress in the applications of physical techniques to the study of carbohydrates is dealt with in Chapters 23—26. Improvements in instrumentation have allowed structural information to be obtained from the natural-abundance ^{13}C n.m.r. spectra of monosaccharides and the diagnostic potential of spin–lattice relaxation times to be more fully explored (Chapter 23). Seventy or so new crystal and molecular structures of carbohydrate derivatives were published during 1974 (see Chapter 24), providing a wealth of information against which currently-held concepts and the results of theoretical calculations can be tested.

An account of the development of Haworth's concepts of ring conformation and of neighbouring-group effects has appeared.[1] General reviews of recent

[1] H. S. Isbell, *Chem. Soc. Rev.*, 1974, **3**, 1.

developments in the chemistry of monosaccharides [2] and of the total synthesis of monosaccharides [3a] have been published, and other specialized reviews have dealt with the applications of electrochemical [3b] and photochemical [3c] processes to carbohydrates and their derivatives.

The October issue [4] of *Carbohydrate Research* was dedicated to the memory of Professor W. Z. Hassid, and the July issue [5] was dedicated to Dr. H. S. Isbell in honour of his seventy-fifth birthday.

[2] J. S. Brimacombe and L. C. N. Tucker, *Ann. Reports (B)*, 1974, **70**, 431.
[3a] J. K. N. Jones and W. A. Szarek, *Total Synthesis of Natural Products*, 1973, **1**, 1.
[3b] M. Fedoronko, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 107.
[3c] K. Matsuura, Y. Araki, and Y. Ishido, *Kagaku No Ryoiki*, 1973, **27**, 1099 (*Chem. Abs.*, 1974, **80**, 133 706h).
[4] *Carbohydrate Res.*, 1974, Vol. 37.
[5] *Carbohydrate Res.*, 1974, Vol. 35.

2
Free Sugars

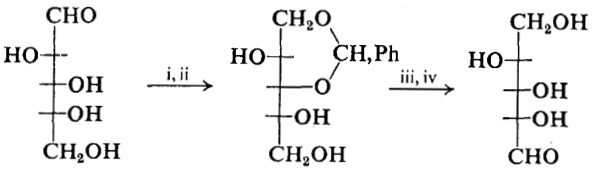
Reviews have appeared on the ionization of carbohydrates in the presence of metal hydroxides and oxides,[6] and on the enolization and oxidation reactions of reducing sugars.[7] Formose sugars have continued to receive attention, and their synthesis and utilization have been reviewed.[8] A unifying mechanism for the formose reaction has been developed based on observed rate phenomena; the mechanism explains why almost any base, regardless of valence, catalyses the formose reaction and the accompanying Cannizzaro reactions of formaldehyde.[9] The same paper reported that $Ca(OH)^+$ is the actual catalytic species for the formose reaction, and another paper [10] has reported that rare-earth hydroxides, especially gadolinium hydroxide, inhibited the reaction. The formose reaction has also been followed potentiometrically; changes in the oxidation–reduction potential curve could be related satisfactorily to the postulated phases of the reaction.[11]

Isolation and Synthesis

Glucose, fructose, 2- and 3-*O*-methylfucose, rhamnose, sedoheptulose, sucrose, mannitol, and laminitol have been identified in ethanolic extracts of the brown seaweed *Desmarestia aculeata*,[12] and the hyaluronate-peptide of vitreous humour has been shown to contain arabinose, fucose, and a 7-deoxyheptose (either 7-deoxy-L-*glycero*-D-*manno*-heptose or 7-deoxy-L-*glycero*-D-*gluco*-heptose).[13]

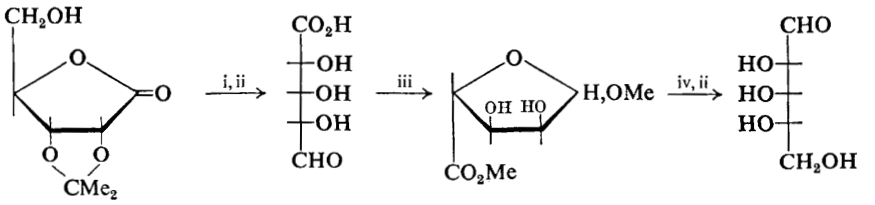
L-Erythrulose (L-*glycero*-tetrulose) has been obtained by the oxidation of erythritol with *Acetobacter suboxydans* [14] and by the degradation of calcium D-*threo*-2,5-hexodiulosonate in either neutral or slightly acidic media.[15] D-Arabinose has been transformed into D-lyxose by the steps shown in Scheme 1, and L-lyxose was similarly obtained from L-arabinose.[16] The same principle was utilized in a synthesis of L-ribose from D-ribono-1,4-lactone (see Scheme 2).[17]

[6] J. A. Rendlemen, jun., in 'Carbohydrates in Solution', A.C.S. Advances in Chemistry Series, No. 117, 1973, p. 51.
[7] H. S. Isbell, in ref. 6, p. 70.
[8] T. Mizuno and A. H. Weiss, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 173.
[9] A. H. Weiss and T. John, *J. Catalysis*, 1974, **32**, 216.
[10] A. A. Morozov and O. E. Levanevskii, *Doklady Akad. Nauk S.S.S.R.*, 1974, **216**, 350.
[11] T. Matsuura, Y. Shigemasa, and C. Sakazawa, *Chem. Letters*, 1974, 713 (*Chem. Abs.*, 1974, **81**, 91 820d).
[12] E. Percival and M. Young, *Carbohydrate Res.*, 1974, **32**, 195.
[13] R. Varma, R. S. Varma, W. S. Allen, and A. H. Wardi, *Carbohydrate Res.*, 1974, **32**, 386.
[14] H. J. Hass and B. Matz, *Annalen*, 1974, 342.
[15] K. Imada, K. Inoue, and M. Sato, *Carbohydrate Res.*, 1974, **34**, C1.
[16] T. A. Giudici and J. J. Griffin, *Carbohydrate Res.*, 1974, **33**, 287.
[17] T. E. Walker and H. P. C. Hogenkamp, *Carbohydrate Res.*, 1974, **32**, 413.



Reagents: i, $NaBH_4$; ii, PhCHO–H^+; iii, DMSO–DCC–CF_3CO_2H; iv, H_3O^+

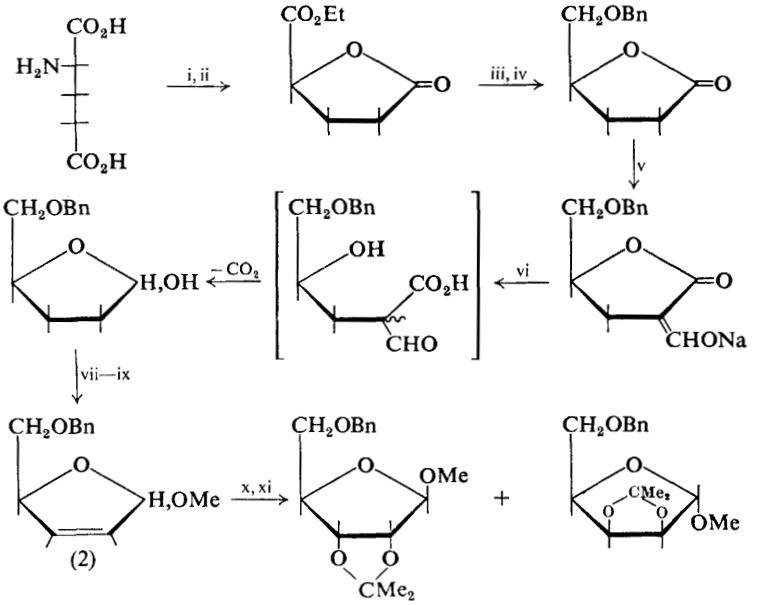
Scheme 1



(1)

Reagents: i, DMSO–DCC–H^+; ii, H_3O^+; iii, MeOH–H^+; iv, $NaAlH_3(OCH_2CH_2OMe)_2$

Scheme 2



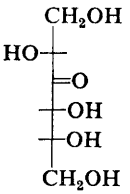
(2)

Reagents: i, HNO_2; ii, EtOH–H^+; iii, $NaBH_4$; iv, BnBr; v, HCO_2Et–NaOEt; vi, H_3O^+; vii, MeOH–H^+; viii, Br_2–Et_2O; ix, NaOMe–MeOH; x, $KMnO_4$; xi, Me_2CO–H^+

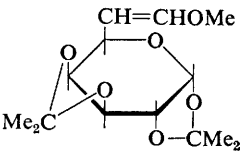
Scheme 3

The latter synthesis was adapted to prepare D-[5-^{13}C]ribose from L-erythrose by way of the enantiomer of the lactone (1).[18] D-Ribose and D-lyxose (as derivatized glycosides) have also been obtained from L-glutamic acid by the procedure shown in Scheme 3, hydroxylation of each of the unsaturated glycosides (2) occurring from the side opposite to the anomeric methoxy-group.[19]

L-Galactose, L-mannose, and L-talose have been obtained from the appropriate 1-deoxy-1-nitro-L-alditols by an improved procedure, oxidative decomposition with hydrogen peroxide in the presence of molybdate ions replacing the classical procedure involving treatment of the sodium salts with dilute sulphuric acid.[20] The epimerization of D-galactose with molybdic acid is claimed to afford a convenient synthesis of D-talose, which was obtained in 16% yield; a small amount (2.4%) of D-gulose was also formed.[21] D-[2-^{3}H]Glucose has been prepared by enzymic methods from D-fructose in tritiated water, and the hexose was sub-

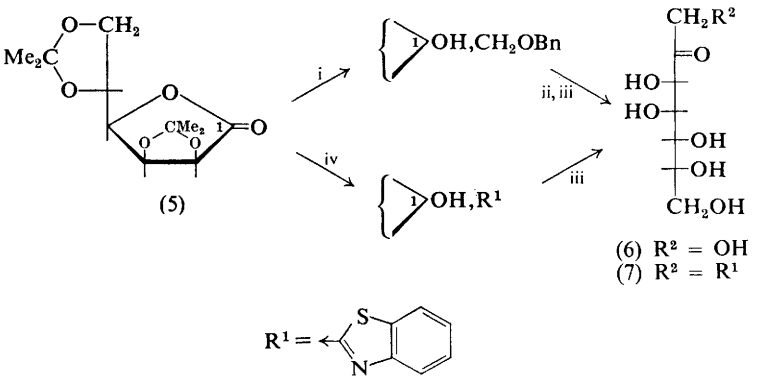


(3)



(4)

sequently converted into D-[2-^{3}H]xylose.[22] Dephosphorylation of the hexose phosphate produced by *Methylococcus capsulatus* has been shown to give D-*arabino*-hex-3-ulose (3) and not D-allulose as previously supposed.[23] Self-condensation of DL-glyceraldehyde 3-phosphate in the presence of ethylenediamine



(5)

(6) R^2 = OH
(7) R^2 = R^1

Reagents: i, $BnOCH_2MgCl$; ii, H_2–Pd; iii, H_3O^+; iv, R^1Li

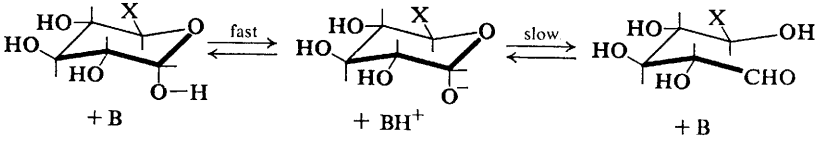
Scheme 4

[18] T. E. Walker, H. P. C. Hogenkamp, T. E. Needham, and N. A. Matwiyoff, *Biochemistry*, 1974, **13**, 2650.
[19] M. Taniguchi, K. Koga, and S. Yamada, *Tetrahedron*, 1974, **30**, 3547.
[20] V. Bilik, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1621.
[21] V. Bilik, W. Voelter, and E. Bayer, *Annalen*, 1974, 1162.
[22] D. W. Harris and M. S. Feather, *J. Org. Chem.*, 1974, **39**, 724.
[23] M. B. Kemp, *Biochem. J.*, 1974, **139**, 129.

has furnished DL-fructose 1,6-diphosphate.[24] A Wittig reagent was used in the preparation of 6-deoxy-D-*galacto*-heptose, by way of the enol ether derivative (4).[25] The D-mannono-1,4-lactone diacetal (5) has been used to prepare D-*manno*-heptulose (6)[26] and a related 1-deoxy derivative (7)[26a] thereof (see Scheme 4).

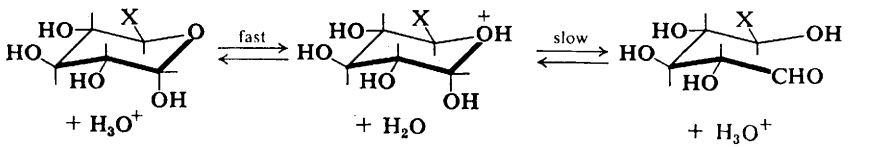
Physical Measurements

In a detailed paper, Capon and Walker have discussed the kinetics and mechanism of the mutarotation of D-xylose and a series of 6-substituted D-glucoses catalysed by the hydroxonium ion, water, and organic bases.[27] Electron-withdrawing substituents at C-6 decreased the rate of mutarotation catalysed by the hydroxonium ion and by water, whereas the rate of the based-catalysed mutarotations was enhanced. On the other hand, electron-withdrawing substituents at C-2 decreased the rate of mutarotation in all cases, except for that of 2-amino-2-deoxy-D-glucose hydrochloride catalysed by lutidine, which occurred at a slightly faster rate than that of 2-deoxy-D-*arabino*-hexose. The authors favour the mechanisms outlined in Scheme 5. A concerted mechanism, in which the ring-



X = H, CH_2OH, Me, *etc.*

Mechanism of base-catalysed mutarotation of aldoses

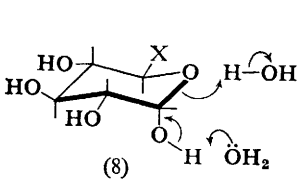


Mechanism of hydroxonium-ion-catalysed mutarotation of aldoses

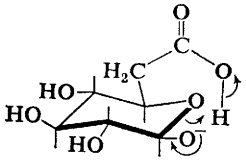
Scheme 5

oxygen atom carries a substantial positive charge in the transition state (8), was proposed for the water-catalysed reaction. Intramolecular catalysis was found in the mutarotations of 6-deoxy-D-*gluco*-hepturonic acid (9) and 6-*O*-(*o*-hydroxyphenyl)-D-glucose (10). Other papers on mutarotation have included a study on trimethylsilylated sugars by g.l.c. and mass spectrometry,[28] kinetic and thermodynamic studies on β-D-arabinopyranose involving catalysis by various amino-

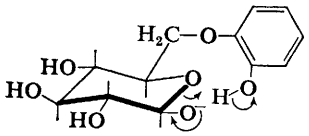
[24] N. Ya. Kozlova and I. V. Mel'nichenko, *Ukrain. khim. Zhur.*, 1974, **40**, 260 (*Chem. Abs.*, 1974, **81**, 4157b).
[25] K. Eklind, P. J. Garegg, B. Lindberg, and A. Pilotti, *Acta Chem. Scand.* (*B*), 1974, **28**, 260.
[26] A. Kampf and E. Dimant, *Carbohydrate Res.*, 1974, **32**, 380.
[26a] Yu. A. Zhdanov, V. G. Alekseeva, and V. N. Formina, *Doklady Akad. Nauk S.S.S.R.*, 1973, **212**, 99.
[27] B. Capon and R. B. Walker, *J.C.S. Perkin II*, 1974, 1600.
[28] I. M. Campbell and R. Bentley, in ref. 6, p. 1.



(8)



(9)



(10)

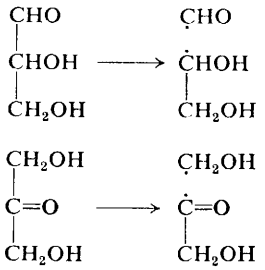
alcohols,[29] and studies on D-glucose in DMF [30] and in water–DMF mixtures;[31] furanose forms (*ca.* 4.5% at 70 °C) were shown to be involved in the mutarotation of D-glucose in DMF. A value of 10.3 kcal mol^{-1} has been calculated for the energy barrier to anomerization of α-D-glucopyranose.[32]

Isotopic exchange equilibria have shown that the binding of the hydroxy-protons of *gem*-diols is tighter than in simple alcohols; similar conclusions were reached for free sugars and, without proof, the effect was ascribed to the hemi-acetal function.[33] However, no control experiments were performed with either vicinal diols or polyhydroxy-systems. The interactions of electrolytes with D-glucitol, D-glucose, glycerol, D-mannitol, and sucrose have been measured by conductometric studies; D-glucose and sucrose were found to associate with the electrolytes, whereas D-glucitol interacted only rarely and D-mannitol and glycerol not at all.[34]

Examination of the volatile products (H_2, D_2, and HD) of γ-irradiated, crystalline mono- and di-saccharides with unlabelled and labelled hydroxy-groups indicated that transfer from exchangeable positions occurred at an early stage of the irradiation.[35] A number of γ-irradiated saccharides emitted light when dissolved in water; trapped free radicals are considered to be responsible for the emission, and an attempt was made to correlate the e.s.r. spectra with the lyoluminescence.[36]

The redox potentials of free radicals formed by the reactions of D-ribose and 2-deoxy-D-*erythro*-pentose with hydroxy-radicals have been studied; at least two radicals, with different redox potentials, were formed at pH 7.[37] An investigation of the photochemistry of glyceraldehyde and 1,3-dihydroxyacetone by CIDNP (Chemically Induced Dynamic Nuclear Polarization) has demonstrated the occurrence of the radical processes shown in Scheme 6. CIDNP signals were not

[29] I. P. Murina, E. I. Klabunovskii, V. A. Pavlov, and E. M. Cherkasova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 333.
[30] A. Reine, J. A. Hveding, O. Kjolberg, and O. Westbye, *Acta Chem. Scand.*, 1974, **B28**, 690.
[31] F. Gram, J. A. Hveding, and A. Reine, *Acta Chem. Scand.*, 1973, **27**, 3616.
[32] M. M. Voronovitskii, A. A. Lugovskoi, and V. G. Dashevskii, *Zhur. strukt. Khim.*, 1974, **15**, 573.
[33] J. F. Mata-Segreda, S. Wint, and R. L. Schowen, *J. Amer. Chem. Soc.*, 1974, **96**, 5608.
[34] S. P. Moulik and D. P. Khan, *Carbohydrate Res.*, 1974, **36**, 147.
[35] G. Lofroth and T. Gejvall, *Acta Chem. Scand.*, 1974, **B28**, 777.
[36] N. A. Atari and K. V. Ettinger, *Radiation Eff.*, 1973, **20**, 135 (*Chem. Abs.*, 1974, **80**, 83 446a).
[37] P. S. Rao and E. Hayon, *J. Amer. Chem. Soc.*, 1974, **96**, 1287.



Scheme 6

shown by tetroses and pentoses.[38] Glycosyl azides have been demonstrated to undergo photolysis with the formation of the corresponding lower aldose (see also Chapter 10).[39]

U.v. absorptions in the region 265—310 nm have been observed during the reactions of formaldehyde with aldoses, ketoses, and alditols in aqueous solutions of sodium hydroxide; in aqueous solutions of calcium hydroxide, absorptions were observed in the region 325—336 nm, and may be due to the formation of complexes with the enol forms.[40]

Theoretical calculations on β-D-glucopyranose using the CNDO/2 method have predicted the most stable conformer ($^4C_1$) to have five hydrogen bonds between the hydroxy-groups and the adjacent oxygen atoms.[41]

The kinetics of oxidation of D-ribose with chloramine-T in alkaline solution have been studied, and the postulated mechanism involves a trimolecular rate-determining step between OCl^-, OH^-, and the anion of β-D-ribose.[42]

Reactions

The molybdate-catalysed epimerization of D-galactose has already been mentioned.[21] Bilik and his colleagues have also reported on related epimerizations of erythrose and threose, to give a 3 : 4 mixture of the two tetroses,[43] and the epimerizations of L-arabinose to L-ribose, of D-xylose to D-lyxose, and of L-xylose to L-lyxose.[44] Equilibrations of D-fructose, D-sorbose, D-tagatose, and D-psicose in the presence of molybdate ions were also examined.[45] The epimerization and degradation of D-glucose in dilute solutions of sodium hydroxide have been studied, and optimum conditions for its degradation to D-arabinose were determined.[46] Radiolysis of frozen, aqueous solutions of hexoses at −78 °C led to isomerization and to the formation of pentoses.[47]

[38] K.-G. Seifert, *Tetrahedron Letters*, 1974, 4513.
[39] J. Plenkiewicz, G. W. Hay, and W. A. Szarek, *Canad. J. Chem.*, 1974, **52**, 183.
[40] T. I. Khomenko and O. V. Krylov, *Kinetika i Kataliz*, 1974, **15**, 625 (*Chem. Abs.*, 1974, **81**, 91 827m).
[41] S. L. Korppi-Tommola and J. J. Lindberg, *Commentat. Phys.-Math. Soc. Sci. Fenn.*, 1973, **43**, 167.
[42] S. P. Mushran, K. C. Gupta, and R. Sanehi, *J. Indian Chem. Soc.*, 1974, **51**, 145.
[43] V. Bilik and L. Stankovic, *Chem. Zvesti*, 1973, **27**, 544.
[44] V. Bilik and J. Caplovic, *Chem. Zvesti*, 1973, **27**, 547.
[45] V. Bilik and K. Tihlarik, *Chem. Zvesti*, 1974, **28**, 106.
[46] K. Koizumi, K. Hashimoto, M. Mitarai, and C. Sawada, *Yakugaku Zasshi*, 1974, **94**, 232 (*Chem. Abs.*, 1974, **80**, 121 227q).
[47] N. K. Kochetkov, L. I. Kudryashov, T. M. Senchenkova, and V. L. Danilov, *Doklady Akad. Nauk S.S.S.R.*, 1973, **213**, 95.

A new crystalline phase of D-glucose has been obtained from aqueous solution; it may be a hydrated form of β-D-glucopyranose, since it was transformed into stable α-D-glucopyranose monohydrate at 32—38 °C.[48] Conditions have been determined for the preparation of ferric-D-fructose and ferric-D-fructose-D-glucose complexes that could be isolated and redissolved to give neutral solutions; studies with labelled substrates showed that no interconversion of D-fructose and D-glucose occurred in the complexes.[49] The yellow colour that accompanies the degradation of sugars with acid has been attributed to further oxidation of 5-(hydroxymethyl)-2-furaldehyde and 2-furaldehyde to γ-unsaturated dicarbonyl compounds, whose broad absorption bands extend into the violet region of the visible spectrum.[50] The conversions of D-[2-^{3}H]xylose and D-[2-^{3}H]glucose with acid into 2-furaldehyde and the 5-(hydroxymethyl) derivative thereof, respectively, involve transfer of hydrogen from C-2 to C-1, since the formyl groups of the products were found to be labelled with tritium.[22]

Intraveneous administration of ^{14}C-labelled D-psicose to rats resulted in its virtual complete excretion in the urine, whereas a large part of D-psicose was metabolized by intestinal micro-organisms following oral feeding.[51]

[48] G. R. Dean, *Carbohydrate Res.*, 1974, **34**, 315.
[49] S. A. Barker, P. J. Somers, and J. Stevenson, *Carbohydrate Res.*, 1974, **36**, 331.
[50] A. M. Taher and D. M. Cates, *Carbohydrate Res.*, 1974, **34**, 249.
[51] R. L. Whistler, P. P. Singh, and W. C. Lake, *Carbohydrate Res.*, 1974, **34**, 200.

3
Glycosides

O-Glycosides

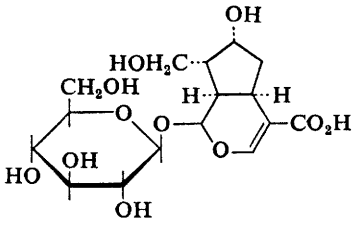
Reviews on flavonoid glycosides (232 references)[52] and on the synthesis of citrus flavonoid glycosides (65 references)[53] have appeared.

Synthesis.— The problems of glycoside synthesis, in particular with methods involving glycosyl halides and 1,2-orthoesters, have been discussed.[54]

The products of Fischer methanolysis of D-galacturonic acid have been monitored by g.l.c. Esterification occurred most rapidly, and was succeeded by the formation in sequence of furanosides and pyranosides. Interestingly, small proportions of the dimethyl acetals of D-galacturono-6,3-lactone and D-galacturonic acid were detected as kinetically controlled products early in the methanolysis.[55] Specific points relating to the Fischer method of glycosidation have been made: acetic acid is recommended instead of hydrogen chloride for glycosidation of the 2-pentuloses,[56] and molecular sieves can usefully be employed to remove the water liberated in the reaction.[56a] It was claimed that methyl α-D-gluco- and -manno-pyranosides can be obtained in *ca.* 89% yield using this modification. However, the preparation of the former glycoside with this efficiency cannot be a one-step operation, since it exists only to the extent of 66% in equilibrium with its isomers. Treatment of sucrose in 30% aqueous ethanol with yeast invertase has afforded a means of obtaining ethyl β-D-fructofuranoside.[57] A sophisticated treatment of the methanolysis of D-glucose has produced a computer program that predicts the yields of the four isomeric glycosides at any stage of the reaction.[58]

It is unusual for sugar derivatives with free hydroxy-groups at C-1 to be used in glycoside synthesis, except in the Fischer reaction. However, 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose has been used to prepare the compound (11) (with boron trifluoride as catalyst),[59] and the condensation of 2-amino-2-deoxy-D-galactose with either D-glucuronic acid or D-mannurono-6,3-lactone in the presence of

[52] H. Wagner, *Fortschr. Chem. org. Naturstoffe*, 1974, **31**, 153.
[53] L. Hoerhammer, G. Aurnhammer, and H. Wagner, *Recent Developments Chem. Natural Carbon Compounds*, 1973, **5**, 29.
[54] G. Wulff and G. Röhle, *Angew. Chem. Internat. Edn.*, 1974, **13**, 157.
[55] K. Larsson and G. Petersson, *Carbohydrate Res.*, 1974, **34**, 323.
[56] L. Stankovič, K. Linek, and M. Fedoroňko, *Carbohydrate Res.*, 1974, **35**, 242.
[56a] M. B. Kozikowski and G. Kupyrszewski, *Roczniki Chem.*, 1973, **47**, 1899.
[57] H. Seaki, Y. Shimada, Y. Ohashi, and E. Ohki, *Sankyo Kenkyusho Nempo*, 1973, **25**, 135.
[58] D. F. Mowery, *J. Phys. Chem.*, 1974, **78**, 1918.
[59] L. F. Tietze, *Angew. Chem. Internat. Edn.*, 1973, **13**, 763.



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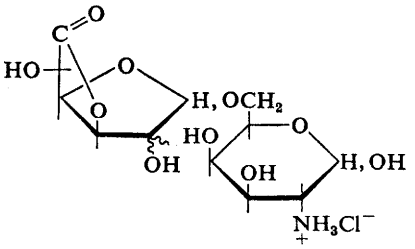
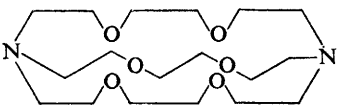


(12)

hydrochloric acid gave the 6-linked disaccharide derivatives (12).[60] In the absence of an acid catalyst, *N*-linked disaccharides were formed (see Chapter 10). Similarly, treatment of methyl α-D-glucopyranoside in *p*-dioxan with an excess of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose in the presence of perchloric acid and Drierite gave a mixture of di- and tri-saccharides, demonstrated to contain mainly 1,1- and 1,6-linked disaccharides, and 1,2- and 1,6-linked trisaccharides.[61]

Acylated glycosyl halides have continued to be used extensively as glycosylating agents. Bromides are usually considered to have the most suitable characteristics for glycosylations, whereas iodides have been used infrequently. However, it has been shown that benzoylated glycopyranosyl iodides, prepared *in situ* from the chlorides, reacted with the lower alcohols in acetonitrile in the presence of 2,6-lutidine to give mixtures of glycosides containing a high proportion of α-glycosides.[62] It was suggested that this modification could be useful for the synthesis of oligosaccharides. Another interesting innovation has utilized crown ethers and related compounds to help solubilize the salts used in the reactions; thus, the use of dibenz-[18]-crown-6 with silver nitrate facilitated the synthesis of β-glycosides from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide.[63] However, when the bicyclic aminopolyether (13) was used in conjunction with silver



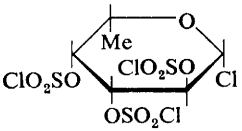
(13)

nitrate, the products contained substantial proportions of glycosyl nitrates (especially with sterically hindered alcohols).[64]

A report on the use of mercury(II) bromide and mercury(II) oxide in the preparation of alkyl β-D-galactopyranosides and β-D-xylopyranosides has appeared,[65] and the tri(chlorosulphated) glycosyl chloride (14) (obtainable in 47% yield directly from L-fucose) has been advocated for the synthesis of α-L-fucopyranosides.[66] Simple α-glycosides may be prepared from (14) by solvolytic methods;

[60] A. Klemer, G. Müller, and A. Ludwig, *Carbohydrate Res.*, 1974, **33**, 263.
[61] A. Klemer and B. Kraska, *Carbohydrate Res.*, 1974, **32**, 400.
[62] F. J. Kronzer and C. Schuerch, *Carbohydrate Res.*, 1974, **34**, 71.
[63] A. Knochel, G. Rudolph, and J. Thiem, *Tetrahedron Letters*, 1974, 551.
[64] A. Knochel and G. Rudolph, *Tetrahedron Letters*, 1974, 3739.
[65] L. R. Schroeder, K. M. Counts, and F. C. Haigh, *Carbohydrate Res.*, 1974, **37**, 368.
[66] M.-E. Rafestin, D. Delay, and M. Monsigny, *Canad. J. Chem.*, 1974, **52**, 210.

the *p*-nitrobenzyl glycoside was also obtained in moderate yield, indicating that complex glycosides can be synthesized in this way. It has been found that β-glycosides of 2-acylamido-2-deoxy-D-glucopyranoses can best be prepared using the glycosyl halides and benzyl alcohol in a two-phase system.[67] Other



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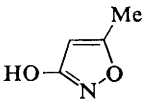


(15)

2-acylamido-compounds to have been reported are the *p*-nitrophenyl glycosides of 2-deoxy-2-(*N*-phthaloylglycylamido)-β-D-glucopyranose and the *N*-phthaloyl-alanylamido-analogue.[68] *p*-Nitrophenyl β-D-chito-bioside and -trioside have also been prepared.[69] Treatment of the isoxazole (15) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide gave mainly the *O*-linked β-glycoside together with a proportion of the corresponding *N*-glycoside.[70]

In the area of dissacharide synthesis, methods of preparing α-D-glucopyranosides and -galactopyranosides are still of considerable interest, and 3,4,6-tri-*O*-acetyl-2-*O*-trichloroacetyl-β-D-galactopyranosyl and -glucopyranosyl chlorides[71] and 2-*O*-benzyl-3,4,6-tri-*O*-(*p*-nitrobenzoyl)-β-D-glucopyranosyl bromide[72, 72a] have been employed for this purpose.

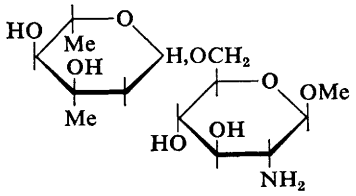
1,2,3,6-Tetra-*O*-acetyl-α- and -β-D-glucopyranoses, for use in Koenigs–Knorr reactions, have been prepared from 4,6-*O*-ethylidene-D-glucopyranose, a key reaction involving replacement of the labile 4-*O*-(1-acetoxyethyl) group with the more stable nitrate group (see p. 44).[73]

Appreciable attention has been given to 6-deoxyhexose disaccharides over the past year, and the preparations of the following disaccharides containing L-rhamnose have been reported: 4-*O*-α-L-rhamnopyranosyl-L-rhamnose,[74] 2-, 3-, and 4-*O*-β-D-glucopyranosyl-L-rhamnose,[75] 4-*O*-α-D-glucopyranosyl-L-rhamnose,[72a] 2-*O*-β-D-galactopyranosyl-L-rhamnose,[75] 3-*O*-β-D-galactopyranosyl-L-rhamnose,[76] 4-*O*-β-D-galactopyranosyl-L-rhamnose,[76, 77] 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnose,[76] and 4-*O*-α-D-mannopyranosyl-L-rhamnose[78] and the β-linked isomer.[79] By contrast, all the L-fucose-containing disaccharides that have been described have L-fucose as the non-reducing

[67] W. D. Rhoads and P. H. Gross, *Z. Naturforsch.*, 1973, **28b**, 647.
[68] M. G. Vafina and N. V. Molodtsov, *Carbohydrate Res.*, 1974, **32**, 161.
[69] F. M. Delmotte and M. L. P. Monsigny, *Carbohydrate Res.*, 1974, **36**, 219.
[70] H. Saeki, Y. Shimada, and T. Murakami, *Sankyo Kenkyusho Nempo*, 1973, **25**, 131 (*Chem. Abs.*, 1974, **80**, 121 267c).
[71] B. Helferich, W. M. Müller, and S. Karbach, *Annalen*, 1974, 1514.
[72] J. M. Berry and G. G. S. Dutton, *Carbohydrate Res.*, 1974, **38**, 339.
[72a] J. M. Berry and G. G. S. Dutton, *Canad. J. Chem.*, 1974, **52**, 681.
[73] D. M. Hall, T. E. Lawler, and B. C. Childress, *Carbohydrate Res.*, 1974, **38**, 359.
[74] G. W. Bebault, G. G. S. Dutton, and C. K. Warfield, *Carbohydrate Res.*, 1974, **34**, 174.
[75] R. R. King and C. T. Bishop, *Canad. J. Chem.*, 1974, **52**, 3913.
[76] R. R. King and C. T. Bishop, *Carbohydrate Res.*, 1974, **32**, 239.
[77] G. M. Bebault, G. G. S. Dutton, and N. A. Funnell, *Canad. J. Chem.*, 1974, **52**, 3844.
[78] G. M. Bebault and G. G. S. Dutton, *Canad. J. Chem.*, 1974, **52**, 678.
[79] G. M. Bebault and G. G. S. Dutton, *Carbohydrate Res.*, 1974, **37**, 309.

component: *viz.* methyl 3-*O*-α-L-fucopyranosyl-α-L-fucopyranoside,[80] 2-*O*-α- and -β-L-fucopyranosyl-D-galactoses,[81] 6-*O*-α-L-fucopyranosyl-D-galactose,[82] and 2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-D-glucose.[83]

Other disaccharide derivatives to have been prepared are 2-acetamido-2-deoxy-6-*O*-α- and -β-D-xylopyranosyl-D-glucopyranoses [84] (prepared using 2,3,4-tri-*O*-chlorosulphonyl-β-D-xylopyranosyl chloride and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl chloride, respectively) and the L-mycarose-containing compounds (16) and (17).[85]



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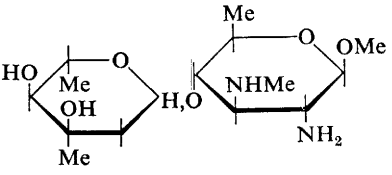
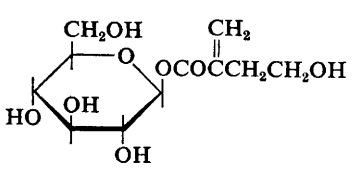
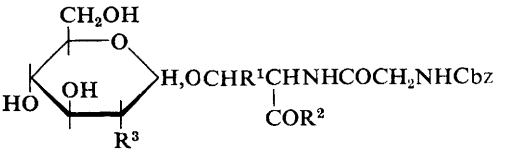


(17)

Among oligosaccharides synthesized were β-D-mannopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 3)-D-galactose, the repeating unit of a polysaccharide found in *Salmonella anatum*,[86] and glycosides of hydroquinone having up to four 1,6-linked β-D-glucopyranose residues in the carbohydrate moiety.[87] A series of β-glycosides of digitoxigenin has been prepared by Koenigs–Knorr condensation of the appropriate derivatives of D-glucose, gentiobiose, and gentiotriose.[88] Arctigenin 4′-β-gentiobioside has also been synthesized,[89] and other glycosylated natural products to be prepared included the plant antifungal agent tuliposide A (18),[90] mono- and di-D-glucosides of zeaxanthin (3,3′-dihydroxy-ββ-carotene),[91]



(18)

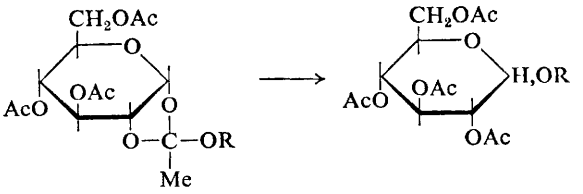


(19) R^1 = H or Me; R^2 = NH_2 or OMe; R^3 = OH or $NHCOCF_3$

[80] M. Dejter-Juszynski and H. M. Flowers, *Carbohydrate Res.*, 1974, **37**, 75.
[81] K. L. Matta, *Carbohydrate Res.*, 1973, **31**, 410.
[82] K. L. Matta, E. A. Z. Johnson, and J. J. Barlow, *Carbohydrate Res.*, 1974, **32**, 418.
[83] K. L. Matta, E. A. Z. Johnson, and J. J. Barlow, *Carbohydrate Res.*, 1974, **32**, 396.
[84] J.-R. Pougny and P. Sinaÿ, *Carbohydrate Res.*, 1974, **38**, 161.
[85] S. Koto, K. Yago, S. Zen, and S. Omura, *Bull. Chem. Soc. Japan*, 1973, **46**, 3800.
[86] N. K. Kochetkov, B. A. Dmitriev, O. S. Chizhov, E. M. Klimov, N. N. Malysheva, V. I. Torgov, Ya. A. Chernyak, and N. E. Bairamova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1386.
[87] K. Takiura, M. Yamamoto, Y. Miyaji, H. Takai, S. Honda, and H. Yuki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2451.
[88] K. Takiura, H. Yuki, Y. Okamoto, H. Takai, and S. Honda, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2263.
[89] S. Nishibe, S. Hisada, and I. Inagaki, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2778.
[90] C. R. Hutchinson, *J. Org. Chem.*, 1974, **39**, 1854.
[91] H. Pfander and M. Holder, *Helv. Chim. Acta*, 1974, **57**, 1641.

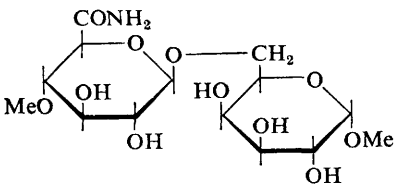
and several glycodipeptides [*e.g.* (19)] containing sugars linked through *O*-glycosidic linkages to L-serine or L-threonine.[92] All the dipeptide derivatives underwent facile β-elimination under alkaline conditions with cleavage of the glycosidic bond.

The orthoester procedure for the synthesis of glycosides has continued to be used, and the alkoxy-group has been shown to influence the ratio of anomeric glycosides formed. Thus, when R = Me (Scheme 7) solvolysis gave mainly the

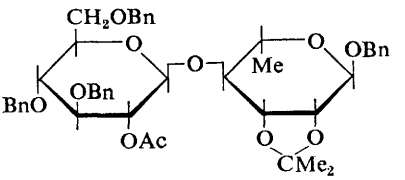


Scheme 7

α-glycoside, whereas a significant proportion of the β-glycoside was produced when R = cyclohexyl.[93] 6-*O*-β-D-Galactofuranosyl-D-galactose has been synthesized by the orthoester procedure,[94] and so too have derivatives of the more complex disaccharide 6-*O*-(3,6-anhydro-β-D-galactopyranosyl)-D-galactose,[95] the uronamide (20),[96] and the D-glucosyl-L-rhamnose disaccharide (21).[97]



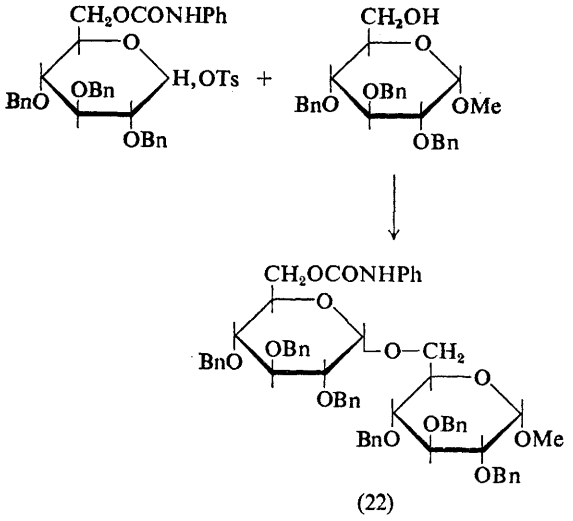
(20)



(21)

An unusually wide range of glycosylating agents have been reported over the past year. Fusion of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose with a catalytic amount of toluene-*p*-sulphonic acid furnished both the α-1,4- and α-1,6-linked disaccharides, whereas similar fusion of the β-tetra-acetate with zinc chloride as catalyst gave higher oligomers and a mannan comprising mainly α-1,6-linkages.[98] Another novel approach has been to use the toluene-*p*-sulphonyloxy-group as a leaving group (Scheme 8); the disaccharide carbamate (22) could then be hydrolysed specifically at the 6′-position to afford a product that was transformed with the same glycosylating agent into an α-linked trisaccharide.[99] Similar steps led

[92] K. Wakabayashi and W. Pigman, *Carbohydrate Res.*, 1974, **35**, 3.
[93] A. F. Bochkov, V. I. Betaneli, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1379.
[94] J.-C. Jacquinet and P. Sinaÿ, *Carbohydrate Res.*, 1974, **34**, 343.
[95] A. F. Bochkov and V. M. Kalinevitch, *Carbohydrate Res.*, 1974, **32**, 9.
[96] A. F. Bochkov and Y. U. Voznyi, *Carbohydrate Res.*, 1974, **32**, 1.
[97] N. K. Kochetkov, B. A. Dmitriev, O. S. Chizhov, E. M. Klimov, N. N. Malysheva, Ya. A. Chernyak, N. E. Bayramova, and V. I. Torgov, *Carbohydrate Res.*, 1974, **33**, C5.
[98] E. O'Brien, E. E. Lee, P. S. O'Colla, and U. Egan, *Carbohydrate Res.*, 1974, **32**, 31.
[99] R. Eby and C. Schuerch, *Macromolecules*, 1974, **7**, 397.



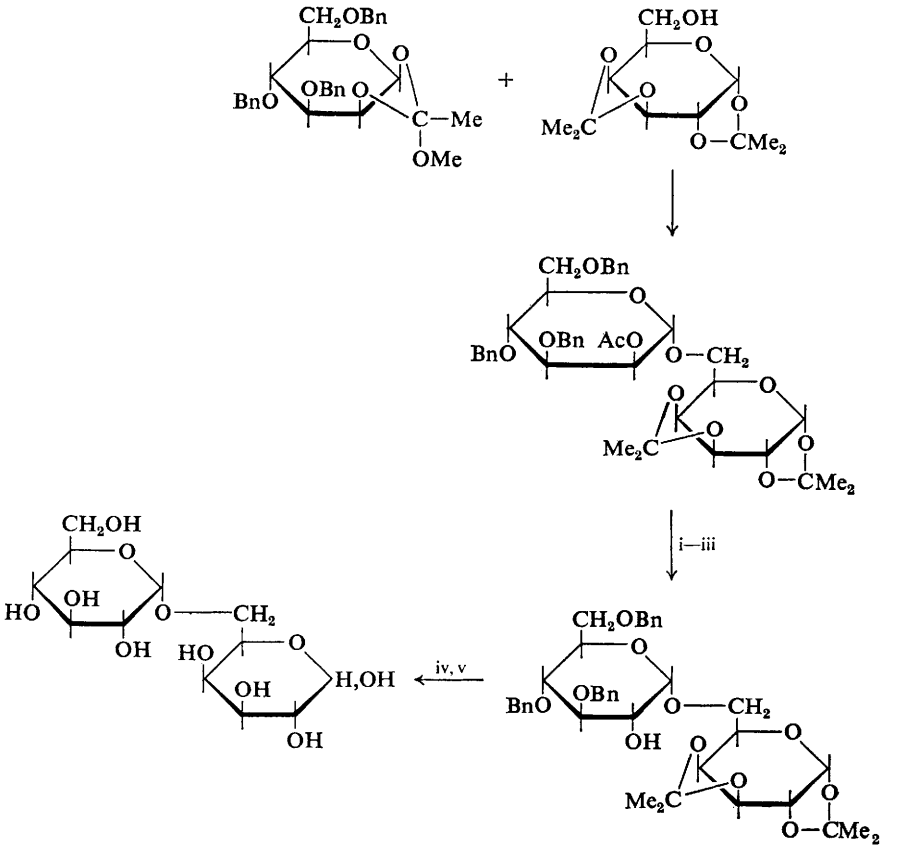
(22)

Scheme 8

to oligomers up to the hexasaccharide. In a preliminary investigation of the use of benzylated 1-*O*-toluene-*p*-sulphonyl-D-glucopyranose derivatives as glycosylating agents, it was shown that the specificity of the reaction depends on the solvent used and on the substituent at C-6; for example, 6-*O*-(*N*-phenylcarbamates) in ether gave high proportions of the α-glycosides.[100] Following work in the D-glucose series (Vol. 7, p. 13) on glycosylating agents with positively charged leaving groups, Kronzer and Schuerch have now studied the solvolyses of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl quaternary ammonium and phosphonium salts (mainly the β-anomers).[101] Methanolysis reactions of the ammonium salts gave good yields of the α-galactoside, but attempts to extend the reaction to the synthesis of disaccharides were unsuccessful. Methanolysis of the phosphonium salts was too slow to be practical.

An approach to glycoside (and oligosaccharide) synthesis that has become increasingly favoured involves the establishment of the glycosidic bond followed by modification of either the aglycone or the glycose moiety. This approach has led to the synthesis of 3-(2-aminoethylthio)propyl glycopyranosides from allyl glycosides,[102] and of 2,3-dithiopropyl β-D-glucopyranoside from the allyl precursor *via* a 2,3-epoxypropyl intermediate.[103] The chirality of the aglycone in 2,3-epoxypropyl β-D-glucopyranoside was determined by correlation with (*R*)-2,3-thiocarbonyldithiopropanol. Several examples of the modification of the glycose moiety, particularly by epimerization at C-2 using an oxidation–reduction sequence, have been reported. Thus, 1,2-*trans*-glycosides can be converted into the *cis*-epimers as illustrated in Scheme 9.[104] The same report has described the

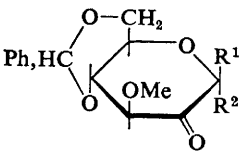
[100] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1974, **34**, 79.
[101] F. J. Kronzer and C. Schuerch, *Carbohydrate Res.*, 1974, **33**, 273.
[102] R. T. Lee and Y. C. Lee, *Carbohydrate Res.*, 1974, **37**, 193.
[103] M. V. Jesudason and L. N. Owen, *J.C.S. Perkin I*, 1974, 1443.
[104] H. B. Boren, G. Ekborg, K. Eklind, P. J. Garegg, A. Pilotti, and C. G. Swahn, *Acta Chem. Scand.*, 1973, **27**, 2639.



Reagents: i, NH_3–MeOH; ii, DMSO–Ac_2O; iii, B_2H_6–THF; iv, CF_3CO_2H–H_2O; v, Pd–C–H_2

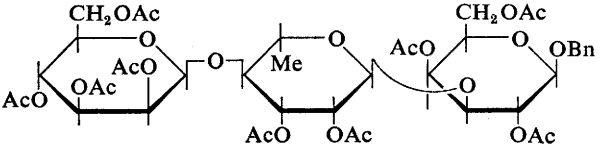
Scheme 9

application of this approach to the preparation of a 6-*O*-β-D-mannopyranosyl-D-galactose analogue. Studies with the model compounds (23) and (24) have shown that reduction with sodium borohydride leads almost exclusively to 1,2-*cis*-products.[105] An elegant application of this procedure has allowed the trisaccharide derivative (25) to be synthesized from the disaccharide (21).[97] Glycoside syn-

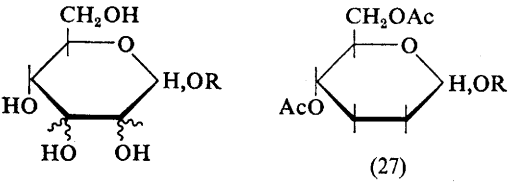


(23) R^1 = H; R^2 = OMe
(24) R^1 = OMe; R^2 = H

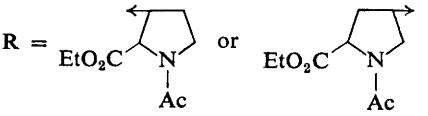
[105] M. Miljković, M. Gligorijević, and D. Miljković, *J. Org. Chem.*, 1974, **39**, 2118.



(25)

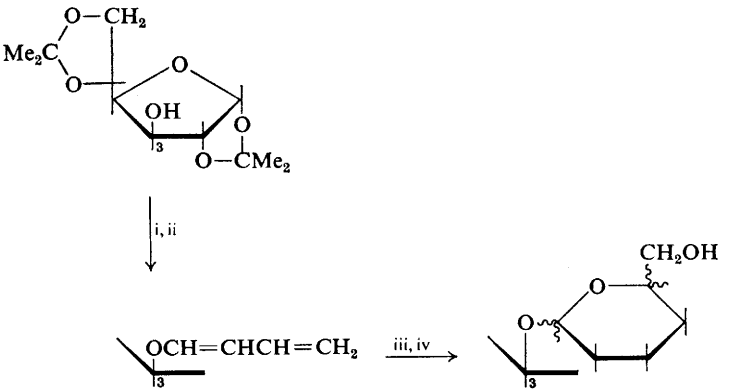


(26) (27)



theses based on acylated glycals also fall into this category; the glycal procedure has been used to synthesize the hydroxyproline glycosides (26) and (27).[106] The glycosyloxyprolines did not undergo β-elimination in base.

A much more elaborate approach to disaccharide synthesis involves the generation of one of the residues by cycloaddition to a dienyl ether of a monosaccharide (see Scheme 10).[107]



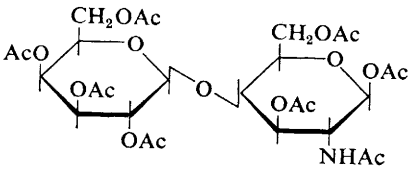
Reagents: i, $Me_2C(OH)C{\equiv}CC{\equiv}C(OH)Me_2$–THF–KOH; ii, H_2–Pd–$BaSO_4$; iii, $BuCO_2CHO$; iv, H_2

Scheme 10

[106] P. D. Feil and J. R. Vercellotti, *Carbohydrate Res.*, 1973, **31**, 311.
[107] S. David, J. Eustache, and A. Lubineau, *J.C.S. Perkin I*, 1974, 2274.

Hydrolysis and Related Reactions.—The hydrolysis of glycosides with acid has continued to attract attention. Painter has examined the effects of anions on the hydrolyses of methyl α- and β-D-glucopyranosides at 70 °C; in aqueous sulphuric, phosphoric, hydrochloric, and hydrobromic acids, k_β/k_α increased with increasing concentration of acid due to the increasing relative stability of the α-anomer. The results were discussed in detail.[108] In an extension of this work, the hydrolyses of cellobiose and maltose were examined, and the effects of inorganic ions on the hydration of the acetal oxygen atoms, as far as they influence the anomeric effect, were considered.[109] It was concluded that the ratio k_β/k_α is associated with the degree of hydration of the disaccharides. De Bruyne has continued his studies in this area and has reported on the hydrolyses of substituted-phenyl α-D-galactopyranosides catalysed by hydrochloric acid. The results seemed to indicate that these acid-catalysed hydrolyses proceeded *via* the cyclic mechanism with protonation of the exocyclic oxygen atom.[110]

An investigation of the hydrolysis of 8-quinolyl β-D-glucopyranoside has been concerned with the significance of intramolecular acid-catalysed hydrolysis. Between pH 1.0 and 5.2, hydrolyses of the free-base form and the *N*-protonated glycoside took place, whereas only specific acid-catalysed hydrolysis of the *N*-protonated species occurred in stronger acid.[111] The evidence suggested that an *A*2 mechanism, rather than an *A*1 mechanism, predominates. Intramolecular metal-ion-catalysis also occurred, especially with copper(II) ions, in the pH region 5.5—6.2, and the rate of reaction was 10^5—10^6 times that of the uncomplexed glycoside. Ferric chloride has been used to convert the disaccharide derivative (28) into the corresponding oxazoline.[112]



(28)

The rates of acetolysis of several disaccharides have been compared; for D-glucobioses, β-linkages were cleaved faster than α-linkages, suggesting anchimeric assistance from the *trans* C-2 acetoxy-group.[113] The mechanisms of the reactions were discussed in detail. The rates of acetolysis in the β-linked D-glucose disaccharides decreased in the order (1 → 6) ⩾ (1 → 3) > (1 → 2) > (1 → 4); for α-linked disaccharides, the order was (1 → 6) ⩾ (1 → 4) > (1 → 3) > (1 → 2).

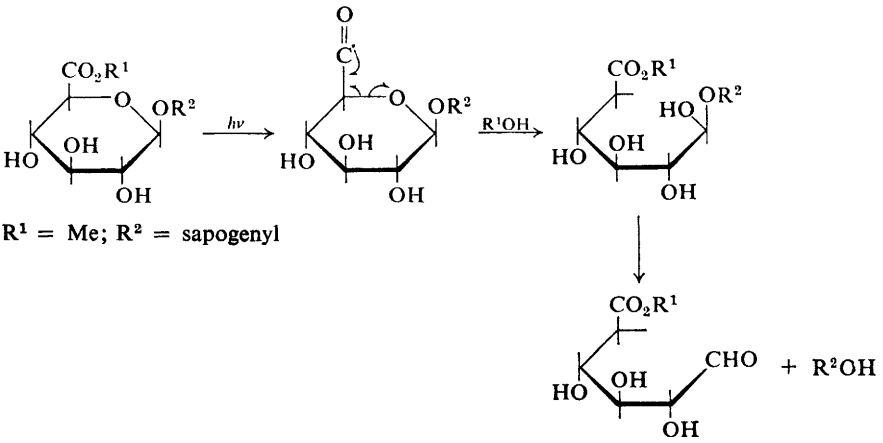
Interest in the hydrolysis of glycosides with alkali has continued. The influence of the concentration of sodium hydroxide on the hydrolysis of *p*-nitrophenyl

[108] T. Painter, *Acta Chem. Scand.*, 1973, **27**, 2463.
[109] T. Painter, *Acta Chem. Scand.*, 1973, **27**, 3839.
[110] C. K. de Bruyne and H. Carchon, *Carbohydrate Res.*, 1974, **33**, 117.
[111] C. R. Clark and R. W. Hay, *J.C.S. Perkin II*, 1973, 1943.
[112] B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 411.
[113] L. Rosenfeld and C. E. Ballou, *Carbohydrate Res.*, 1974, **32**, 287.

β-D-xylopyranoside and its 2-*O*-methyl and 2,3,4-tri-*O*-methyl derivatives, for which participation by an oxyanion at C-2 is precluded, has been examined.[114] Methylation at O-2 reduced the rate by three orders of magnitude, and a full analysis of the results was given.

The alkaline degradation of cellobi-itol, as a model for cellulose, has been studied.[115] At elevated temperatures in the presence of oxygen, D-glucose was liberated following oxidation of the D-glucitol residue. To a lesser extent, the D-glucose moiety was oxidized and D-glucitol liberated in a consecutive reaction. Under the conditions of the experiment, D-glucose was decomposed to a complex mixture of products. A related investigation on the degradation of sucrose in 5% sodium hydroxide solution under nitrogen showed that 2-methyl-, 2,5-dimethyl-, and 2,3,5-trimethyl-*p*-benzoquinone, pyrocatechol, and 3- and 4-methylpyrocatechol were among the products formed.[116]

A photo-induced cleavage of a glycosidic bond has been reported with uronoside esters in the oleanane triterpenoid saponins (Scheme 11); reduction of the



R^1 = Me; R^2 = sapogenyl

Scheme 11

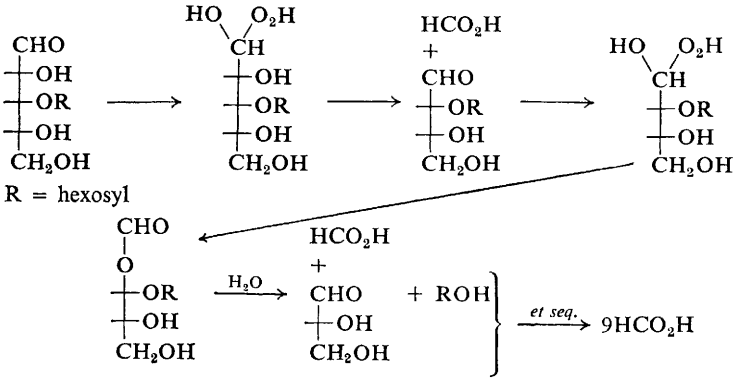
uronic acid ester to the corresponding aldopyranoside completely inhibited the photolysis.[117]

Other Reactions and Features of Glycosides.—Methyl α-D-glycopyranosides have been shown to undergo thermal decomposition in two overlapping stages.[118] At lower temperatures, loss of methanol occurred during inter- and intramolecular condensations, resulting in the formation of glycans and/or 1,6-anhydrohexoses. At higher temperatures, fragmentation of the transglycosylation products occurred. Shafizadeh's group has continued their investigations on thermolytic reactions, and has shown that the thermolysis of phenyl β-D-glucopyranoside

[114] C. K. de Bruyne, F. Van Wijnendaele, and H. Carchon, *Carbohydrate Res.*, 1974, **33**, 75.
[115] O. Samuelson and L. Stolpe, *Acta Chem. Scand.*, 1973, **27**, 3061.
[116] H. Kato, M. Mizushima, T. Kurata, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2677.
[117] I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1339.
[118] G. D. McGinnis and S. Parikh, *Carbohydrate Res.*, 1973, **31**, 183.

in the presence of zinc chloride proceeds in two distinct stages. In the first stage, the Lewis acid facilitated the formation of glucosans; in the second stage, it catalysed the elimination of hydroxy-groups and dehydration.[119] Free radicals formed by γ-irradiation of substituted-phenyl β-D-glucopyranosides in the crystalline state and in frozen solutions have been examined by e.s.r. spectroscopy, and identification has been made of substituted cyclohexadienyl radicals and radicals derived from the D-glucopyranosyl moiety.[120]

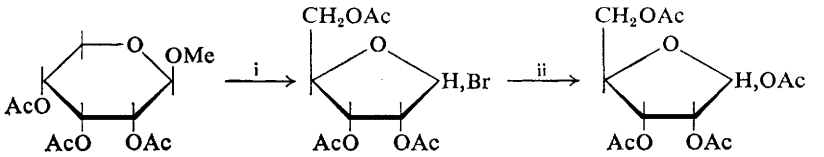
Alkaline hydrogen peroxide was found to degrade completely reducing disaccharides to formic acid by way of formate ester intermediates (see Scheme 12).[121]



Scheme 12

An unusual ring-contraction has been detected following treatment of acetylated methyl glycopyranosides with hydrobromic acid in acetic acid.[122] In addition to the expected pyranosyl bromides, large proportions of furanosyl bromides, which in some cases were the major products, were detected (Scheme 13).

Lobry de Bruyne transformation of panose has yielded 'panulose' [*O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-fructose].[123] Melibiose



Reagents: i, HBr–AcOH; ii, Ac_2O–$ZnBr_2$

Scheme 13

[119] Y.-Z. Lai and F. Shafizadeh, *Carbohydrate Res.*, 1974, **38**, 177.
[120] P. J. Baugh, K. Kershaw, G. O. Phillips, and M. G. Webber, *Carbohydrate Res.*, 1973, **31**, 199.
[121] H. S. Isbell and R. G. Naves, *Carbohydrate Res.*, 1974, **36**, C1.
[122] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1974, **B28**, 1041.
[123] K. Kainuma, K. Sugawara-Hata, and S. Suzuki, *Stärke*, 1974, **26**, 274 (*Chem. Abs.*, 1974, **81**, 120 885m).

underwent epimerization at C-2 on heating with an aqueous solution of molybdic acid, whereas lactose and αα-trehalose were unaffected.[124]

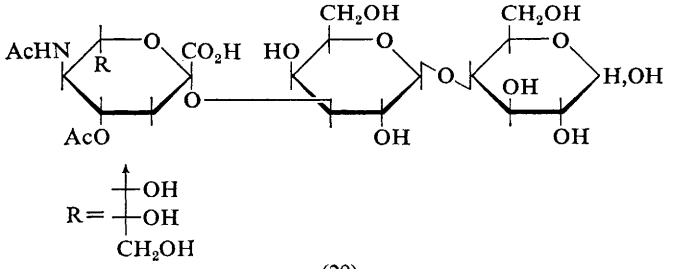
The damage caused to crystalline disaccharides by γ-radiation did not involve cleavage of the glycosidic bond, suggesting that this bond is not responsible for the origin and/or trapping of the radicals.[124a]

Studies of a series of methyl glycosides by ^{13}C n.m.r. spectroscopy are referred to in Chapter 23.

Natural Products.—As in previous volumes, no attempt has been made to produce full coverage in this section.

2-*O*-β-D-Glucopyranosylglycerol and the 1-*O*-acetyl derivative have been isolated from *Lilium longiflorum*.[125]

A new trisaccharide, 6-*O*-β-D-galactopyranosyl-lactose, has been found in human milk,[126] and the sialyl-lactose (29) has been shown to be the main carbohydrate in the milk of the echidna, an Australian egg-laying mammal.[127]



(29)

S-Glycosides

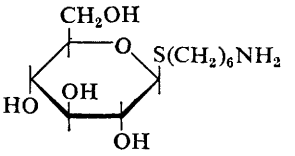
The use of 1-thioglycosides in studies of enzyme inhibition and in affinity chromatography has led to the preparation of several new compounds, *viz.* *p*-nitrophenyl and *p*-aminophenyl 1-thio-α- and -β-L-fucopyranosides,[128] the corresponding 1-thioglycosides of 2-acetamido-2-deoxy-β-D-glucopyranose and -galactopyranose,[129] β-D-galactopyranose, β-D-fucopyranose, and α-D-mannopyranose,[130] and *p*-nitrobenzyl 1-thio-β-chito-bioside and -trioside.[69]

The hydrolyses of 6-purinyl 1-thio-β-D-glucopyranoside with emulsin and acid have been studied in detail (the latter reaction appears to proceed by the *A*1 mechanism),[131] and the syntheses of β-D-glucopyranosides of 1-(4-hydroxyphenyl)- and 1-(4-methoxyphenyl)-5-mercaptotetrazole have been described.[132]

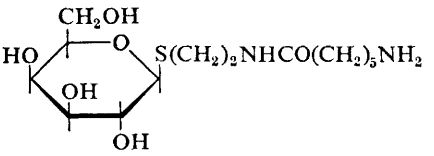
[124] W. Voelter and H. Bauer, *Tetrahedron Letters*, 1974, 3597.
[124a] G. Löfroth and T. Gejvall, *Acta Chem. Scand.* (*B*), 1974, **28**, 829.
[125] M. Kaneda, K. Mizutani, Y. Takahashi, G. Kurono, and Y. Nishikawa, *Tetrahedron Letters*, 1974, 3937.
[126] K. Yamashita and A. Kobata, *Arch. Biochem. Biophys.*, 1974, **161**, 164.
[127] M. Messer, *Biochem. J.*, 1974, **139**, 415.
[128] M. L. Chawla and O. P. Bahl, *Carbohydrate Res.*, 1974, **32**, 24.
[129] C. S. Jones, R. H. Shah, D. J. Kosman, and O. P. Bahl, *Carbohydrate Res.*, 1974, **36**, 241.
[130] R. H. Shah and O. P. Bahl, *Carbohydrate Res.*, 1974, **32**, 15.
[131] L. R. Fedor and B. S. R. Murty, *J. Amer. Chem. Soc.*, 1973, **95**, 8410.
[132] G. Wagner, B. Dietzsch, and G. Fischer, *Pharmazie*, 1974, **29**, 95.

A number of 1-thio-β-D-aldopyranosides (of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose) with aglycones containing a terminal amino-group [*e.g.* (30) and (31)] have been prepared for subsequent attachment to solid matrices.[133, 134]

The photolysis of glycosyl phenyl sulphones is noted immediately below.



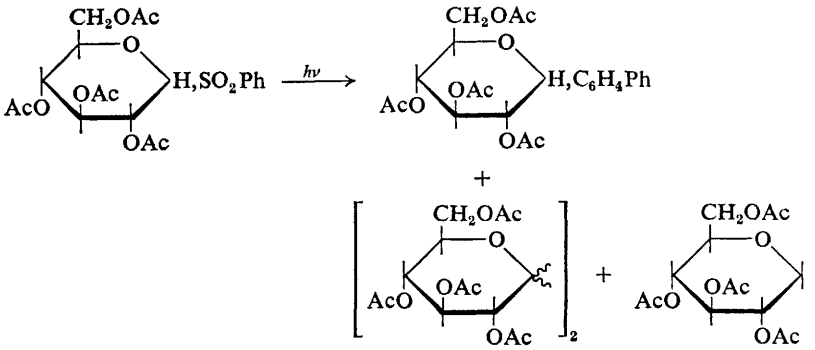
(30)



(31)

C-Glycosides

The photolytic decomposition of α- and β-D-glucopyranosyl phenyl sulphone acetates in benzene yielded a number of products, including those illustrated in Scheme 14. The free-radical mechanism proposed for the formation of products,

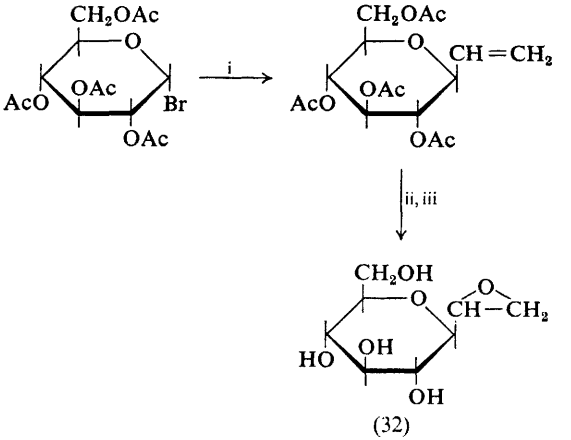


Scheme 14

which included *C*-glycosides, was supported by studies conducted in hexadeuteriated benzene.[135] Phenyl 2,3,5,6-tetra-*O*-acetyl-α-D-glucofuranosyl sulphone likewise gave the anomeric glycosylbiphenyls and αα-, αβ-, and ββ-linked disaccharides.[136]

Russian workers have reported the preparation of the diastereoisomeric *C*-glycosyl-oxirans (32) (Scheme 15)[137, 137a] and (33),[137a] both of which inhibited sweet-almond β-glucosidase. The natural occurrence of *C*-bonded D-ribofuranosyl nucleosides has stimulated further synthetic work in this area.

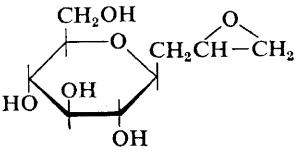
[133] S. Chipowsky and Y. C. Lee, *Carbohydrate Res.*, 1973, **31**, 339.
[134] R. T. Lee and Y. C. Lee, *Carbohydrate Res.*, 1974, **34**, 151.
[135] P. M. Collins and B. R. Whitton, *J.C.S. Perkin I*, 1974, 1069.
[136] P. M. Collins and B. R. Whitton, *Carbohydrate Res.*, 1974, **36**, 293.
[137] S. D. Shiyan, M. L. Shulman, and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2386.
[137a] M. L. Shulman, D. Shiyan, and A. Ya. Khorlin, *Carbohydrate Res.*, 1974, **33**, 229.



(32)

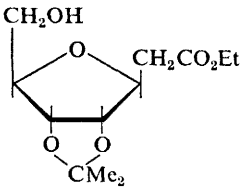
Reagents: i, CH_2=CHMgBr; ii, peracid–MeCN; iii, NaOMe

Scheme 15

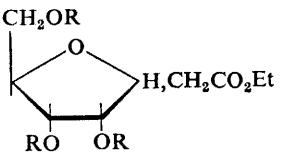


(33)

Hanessian's [138] and Buchanan's groups [139] have applied the Wittig reaction to D-ribofuranose derivatives to obtain the *C*-ribofuranosyl derivatives of ethyl acetate [(34)—(36)] for possible elaboration to *C*-nucleosides. Buchanan and his co-workers have also provided details (*cf.* Vol. 6, p. 27) of the preparation of the

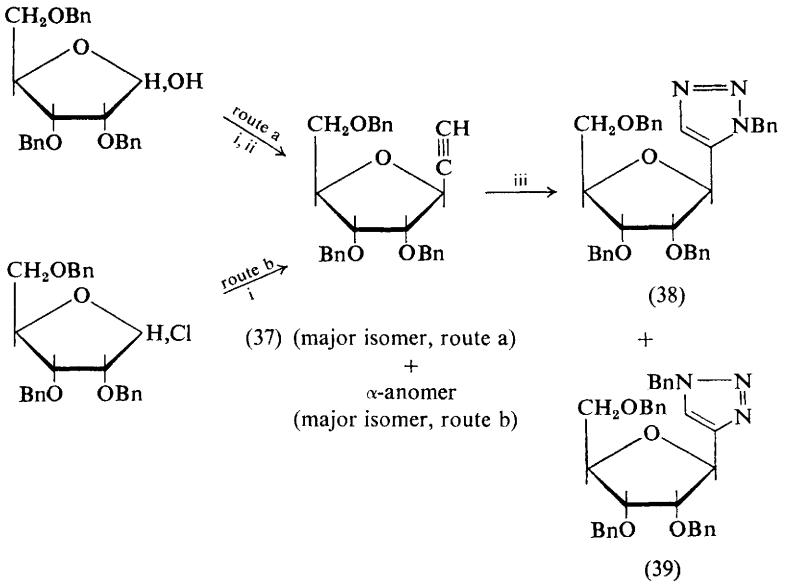


(34)



(35) R = Bz
(36) R = Bn

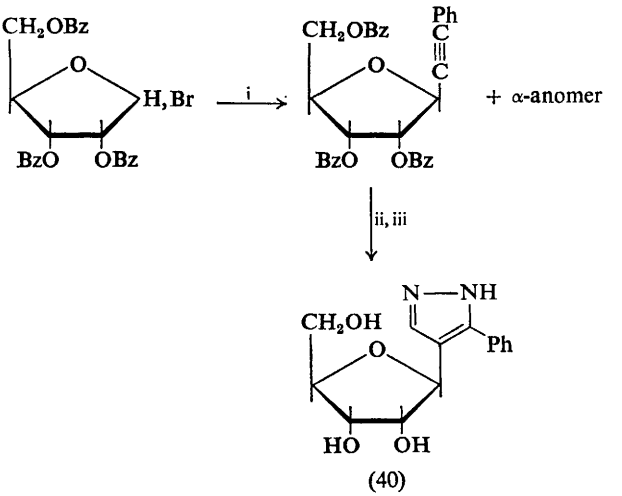
[138] S. Hanessian, T. Ogawa, and Y. Guindon, *Carbohydrate Res.*, 1974, **38**, C12.
[139] J. G. Buchanan, A. R. Edgar, M. J. Power, and P. D. Theaker, *Carbohydrate Res.*, 1974, **38**, C22.



Reagents: i, CH≡CMgBr; ii, TsCl–py; iii, BnN_3

Scheme 16

acetylenic sugar (37) and its conversion into the isomeric *C*-nucleosides (38) and (39) (Scheme 16).[140] A related paper has described the reaction of ethynyl-

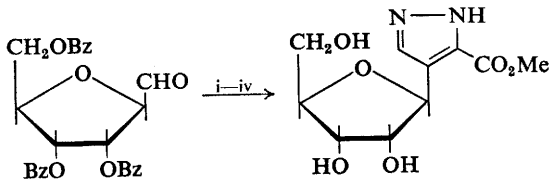


Reagents: i, $(PhC{\equiv}C)_2Hg$; ii, CH_2N_2; iii, NH_3–MeOH

Scheme 17

[140] J. G. Buchanan, A. R. Edgar, and M. J. Power, *J.C.S. Perkin I*, 1974, 1943.

magnesium bromide with 2,3-*O*-isopropylidene-D-ribose and 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, and the syntheses of glycofuranosylethynes related to (37).[141] Similar approaches, incorporating the 1,3-dipolar addition of diazomethane to acetylenic sugars, have permitted access to the *C*-pyrazole derivatives (40) (Scheme 17)[142] and (41) (Scheme 18).[143] Tronchet's group has



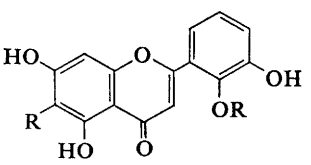
(41)

Reagents: i, $Ph_3P{=}CHCO_2Me$; ii, CH_2N_2; iii, Cl_2; iv, NaOMe–MeOH

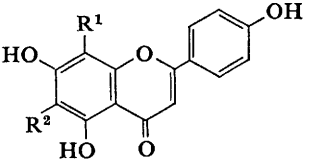
Scheme 18

described two methods for the synthesis of terminal acetylenic sugars, both involving extension of the carbon chain by a single unit.[144]

The *C*-glycosides (42)[145] and (43)[146] have been isolated from plants, and the structure of the di-*C*-glycoside (43) was confirmed by synthesis.



(42) R = β-D-glucopyranosyl



(43) R^1 = β-D-glucopyranosyl; R^2 = α-L-arabinopyranosyl

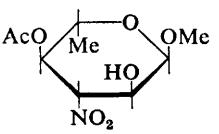
C-Glycosyl antibiotics are referred to in Chapter 20 and conformational features of β-pseudouridine are mentioned in Chapter 23.

[141] J. G. Buchanan, A. D. Dunn, and A. R. Edgar, *Carbohydrate Res.*, 1974, **36**, C5.
[142] K. Arakawa, T. Miyasaka, and N. Hamamichi, *Chem. Letters*, 1974, 1305.
[143] H. P. Albrecht, D. B. Repke, and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 2176.
[144] J. M. J. Tronchet, A. Gonzalez, J.-B. Zumwald, and F. Perret, *Helv. Chim. Acta*, 1974, **57** 1505.
[145] K. Hostettman and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1974, **57**, 204.
[146] M.-C. Biol, M.-L. Bouillant, G. Planche, and J. Chopin, *Compt. rend.*, 1974, **279**, *C*, 409.

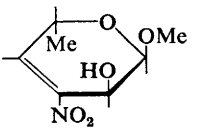
4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—The use of sodium hydride and either methyl iodide or dimethyl sulphate in THF at 50 °C has been advocated for the efficient methylation of alcohols and glycols.[147] Diazomethane and boron trifluoride have been used to methylate such nitro-sugars as (44) and (45) without interference by the nitro-group.[148]



(44)



(45)

The interesting question of the relative rates of alkylation (and acylation) of the hydroxy-groups of α-diols has been examined, and the ratio of the various rate constants allowed the optimum yields of mono-substituted products to be calculated.[149]

A potentially useful method for effecting demethylation without cleavage of glycosidic bonds has used lithium and ethylamine.[150] Isopropylidene and cyclohexylidene groups were shown to be stable under the conditions of demethylation, but benzylidene and benzyl ether groups were cleaved. It will be interesting to see whether the general utility of this reaction can be substantiated and whether a mechanistic rationale can be developed.

The degradation of 3,4-di- and 3,4,6-tri-*O*-methyl-D-glucopyranoses and 3,4,6-tri-*O*-methyl-D-galactopyranose with sodium hydroxide–sodium borohydride mixtures has shown that reduction is faster than β-elimination.[151] G.l.c.–mass spectrometry of the TMS derivatives has been used to analyse the reaction products.

Several reports on the synthesis of specific methyl ethers have appeared. 2-*O*-Methyl-[152] and 2,4- and 3,4-di-*O*-methyl-D-xyloses[153] have been obtained

[147] C. A. Brown, D. Barton, and S. Sivaram, *Synthesis*, 1974, 434.
[148] H. H. Baer and C.-W. Chiu, *Carbohydrate Res.*, 1973, **31**, 347.
[149] J. Staněk, jun., P. Chuchvalec, K. Čapek, K. Kefurt, and J. Jarý, *Carbohydrate Res.*, 1974, **36**, 273.
[150] C. Minneret, J. C. Florent, I. Kabore, and Khong-Huu Qui, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1974, **1**, 161.
[151] G. O. Aspinall and S. C. Tam, *Carbohydrate Res.*, 1974, **38**, 71.
[152] J. Hirsch and P. Kováč, *Chem. Zvesti*, 1973, **27**, 816.
[153] P. Kováč and J. Hirsch, *Chem. Zvesti*, 1973, **27**, 668.

by standard procedures; *e.g.* the 2,4-di-*O*-methyl ether was prepared from 3-*O*-benzyl-D-xylose by methylation and debenzylation. A synthesis of 2,4-di-*O*-methyl-L-arabinose is noted in Chapter 6, and that of 2,3,5-tri-*O*-methyl-D-glucofuranose has been achieved from *N*-acetyl-α-D-glucofuranosylamine *via* the 6-trityl ether.[153a] Preparations of all the possible products of partial methylation of phenyl β-D-glucopyranoside have been reported, together with their characterization by ^{1}H n.m.r. spectroscopy.[154] In connection with structural studies of bacterial polysaccharides, the 3,4,6-tri-*O*-methyl ethers of D-glucopyranose, D-galactopyranose, and D-mannopyranose have been prepared from the corresponding 1,3,4,6-tetra-acetates, following conversion into the 2-*O*-tetrahydropyranyl derivatives.[155] Several methyl ethers of 2-deoxy-2-(*N*-methylacetamido)-D-glucose [156] and -L-gulose [156a] have been separated by g.l.c. and their structures were assigned by mass spectrometric analysis of the derived alditol peracetates. In the D-galactose series, the 3,6-di-*O*-methyl ether has been obtained by opening of a terminal epoxide, [157] and syntheses of 3,4-di- and 3,4,6-tri-*O*-methyl-D-mannopyranose,[158] and 6-mono- and 3,6- and 4,6-di-*O*-methyl ethers of methyl 2-acetamido-2-deoxy-α-D-mannopyranoside have been reported.[159]

In the deoxyhexose series, 2,3-di-*O*-methyl-D-fucose [160] and 3- and 4-mono- and 3,4-di-*O*-methyl ethers of 2-amino-2,6-dideoxy-L-glucose [161] have been prepared; the dimethylated derivative was obtained from 3,4-di-*O*-methyl-L-rhamnal by way of the addition of nitrosyl chloride, which also provided access to the 4-*O*-methyl ether. Perry and his colleagues have also described the corresponding methylated derivatives of 2-amino-2,6-dideoxy-D-galactose in connection with structural studies of polysaccharides.[162] Because degradation by β-elimination accompanies the methylation of uronic acid derivatives, the 2-, 3-, 4-, 2,3-di-, 2,4-di-, and 3,4-di-*O*-methyl ethers of methyl (methyl α-D-glucopyranosid)uronate have been prepared by the oxidation of appropriate *O*-benzyl-*O*-methyl derivatives of methyl α-D-glucopyranoside having the 6-position unsubstituted.[163] Subsequent esterification and debenzylation furnished the required products.

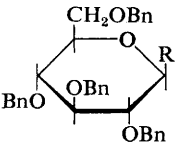
In the alditol series, 2,3-di- and 2,3,6-tri-*O*-methyl-D-mannitol have been prepared by standard procedures.[164] The methylation of nucleosides and investigations of the vicinal ^{13}C–^{1}H couplings of methylated sugars are referred to in Chapters 21 and 23, respectively.

Substituted Alkyl Ethers.—Benzyl trifluoromethanesulphonate has been shown to be a powerful benzylating agent, and it has been used with 1,3,4,6-tetra-*O*-acetyl-α-D-galacto- and -gluco-pyranoses to obtain the 2-*O*-benzyl ethers in good

[153a] M. E. Gelpi and R. A. Cadenas, *Anales Asoc. quim. argentina*, 1973, **61**, 283 (*Chem. Abs.*, 1974, **80**, 96 264q).
[154] P. Nanasi and A. Liptak, *Magyar Kém. Folyóirat*, 1974, **80**, 217 (*Chem. Abs.*, 1974, **81**, 91 880y).
[155] J. M. Berry, Y.-M. Choy, and G. G. S. Dutton, *Canad. J. Chem.*, 1974, **52**, 291.
[156] G. O. H. Swarzmann and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **34**, 161.
[156a] A. Cooke and E. Percival, *Carbohydrate Res.*, 1974, **32**, 383.
[157] J. S. Brimacombe and A. J. Rollins, *Carbohydrate Res.*, 1974, **36**, 205.
[158] F. Seymour, *Carbohydrate Res.*, 1974, **34**, 65.
[159] Nasir-Ud-Din, D. A. Jeanloz, and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **38**, 205.
[160] T. Fujikawa, *Carbohydrate Res.*, 1974, **38**, 325.
[161] M. B. Perry and V. Daoust, *Canad. J. Chem.*, 1974, **52**, 2425.
[162] M. B. Perry and V. Daoust, *Canad. J. Chem.*, 1974, **52**, 3251.
[163] P. Kováč, *Carbohydrate Res.*, 1973, **31**, 323.
[164] C. W. Baker and R. L. Whistler, *Carbohydrate Res.*, 1974, **33**, 372.

yield.[165] Attempts to convert the fully substituted products into the corresponding glycosyl bromides, by treatment with hydrogen bromide in either acetic acid or dichloromethane, were accompanied by debenzylation.

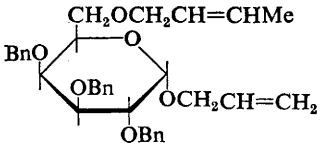
Benzyl ethers have been found among the products of partial hydrogenolysis of 4,6-*O*-benzylidene derivatives using a mixture of lithium aluminium hydride and aluminium trichloride.[154] The ratio of 4- to 6-*O*-benzyl ethers obtained depends on the substituent at C-3. A study of debenzylation in connection with solid-phase oligosaccharide syntheses has examined the reactions of the 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosides (46)—(48) with sodium in liquid ammonia.[166]



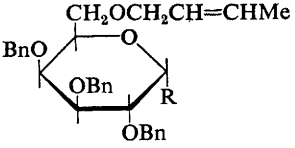
(46) R = SBn
(47) R = SCH_2Bn
(48) R = OBn

With an excess of the reagent, complete debenzylation occurred to yield 1-thio-D-glucose from the 1-thioglycosides (46) and (47), while the *O*-glycoside (48) gave D-glucose. Treatment with limited amounts of the reagent furnished products derived from specific dealkylation at the anomeric centre. The isomeric benzyl tri-*O*-benzyl-α-D-galactopyranosides have been prepared and used in the synthesis of disaccharides,[167] and 2-, 2,6-di-, and 2,3-di-*O*-benzyl-D-galactose[168] and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[169] have been prepared by standard methods. 3,4,6-Tri-*O*-benzyl-2-dibenzylamino-2-deoxy-D-glucopyranose has been derived from allyl 2-benzamido-2-deoxy-β-D-glucopyranoside as a potential intermediate for the synthesis of α-glucosaminides.[167]

In continuing their studies on the use of allyl ethers as protecting groups in carbohydrate chemistry, Gent and Gigg have shown that tris(triphenylphosphine)rhodium(I) chloride is capable of isomerizing the allyl ether group significantly faster than the but-2-enyl group.[170] Thus, compound (49) could be converted specifically into the isomer (50), from which the glycosidic substituent was removed, to afford (51), on treatment with mercury(II) chloride. *p*-Nitro-



(49)

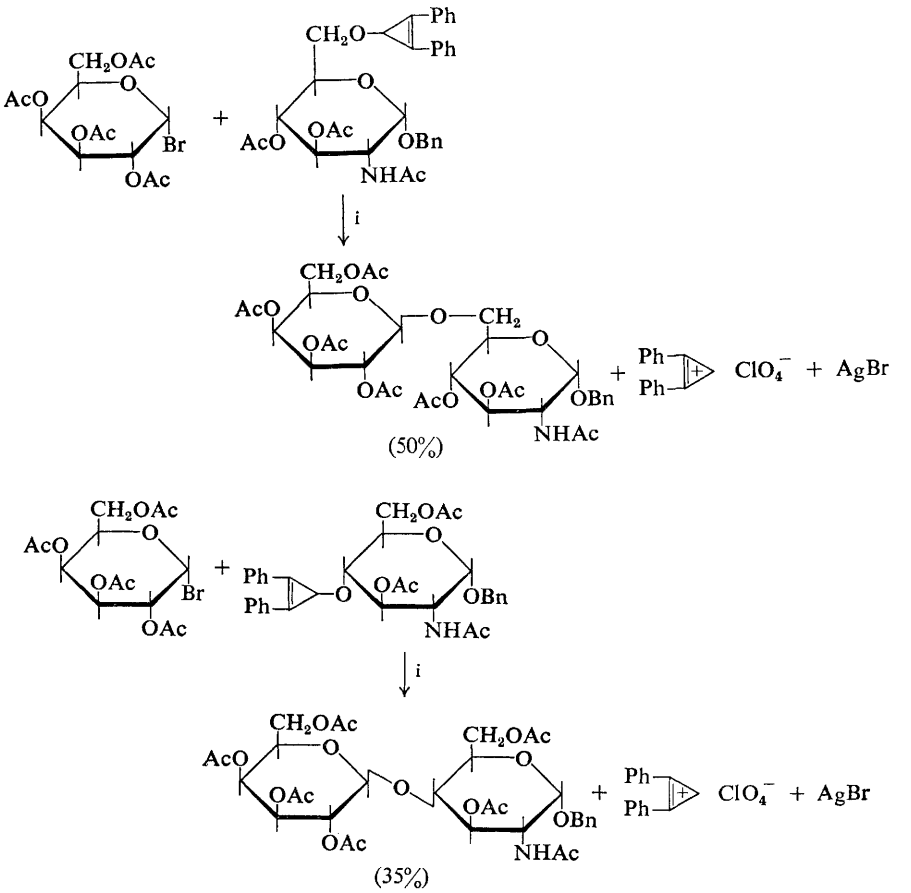


(50) R = OCH=CHMe
(51) R = OH
(52) R = Cl

[165] R. U. Lemieux and T. Kondo, *Carbohydrate Res.*, 1974, **35**, C4.
[166] S. A. Holick and L. Anderson, *Carbohydrate Res.*, 1974, **34**, 208.
[167] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1974, 1446.
[168] J. Schneider, Y. C. Lee, and H. M. Flowers, *Carbohydrate Res.*, 1974, **36**, 159.
[169] J. C. Jacquinet, J.-M. Petit, and P. Sinaÿ, *Carbohydrate Res.*, 1974, **38**, 305.
[170] P. A. Gent and R. Gigg, *J.C.S. Chem. Comm.*, 1974, 277.

benzoylation of (51) gave the 1-ester, which was used to prepare the glycosyl chloride (52).[171] Condensation of (52) with benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside yielded the α-linked disaccharide derivative; the but-2-enyl group could then be removed selectively (KOBu^t–DMSO) to give a product suitable for the synthesis of trisaccharides.

All six tritylmaltose peracetates (6α, 6β, 6′α, 6′β, 6,6′α, and 6,6′β) have been isolated following tritylation of maltose and subsequent acetylation.[171a] The selective tritylation of phenyl α- and β-maltosides has been examined in detail using 1.1 and 2.2 molar equivalents of trityl chloride in pyridine, and the yields of the 6-, 6′-, and 6,6′-di-substituted ethers were recorded.[172]



Reagent: i, $AgClO_4$

Scheme 19

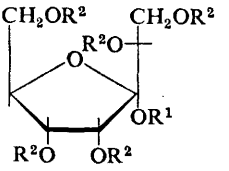
[171] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1974, 1835.
[171a] K. Koizumi and T. Utamura, *Carbohydrate Res.*, 1974, **33**, 127.
[172] K. Takeo, S. Kato, and T. Kuge, *Carbohydrate Res.*, 1974, **38**, 346.

In seeking an ether group which cleaves to form a stable carbonium ion under conditions of glycosylation (like the trityl group), but which can be introduced readily at secondary hydroxy-groups (unlike the trityl group), Russian chemists have successfully utilized the 2,3-diphenyl-2-cyclopropen-1-yl group.[173] The ethers were obtained by treatment of a partially protected glycoside with 2,3-diphenyl-2-cyclopropen-1-ylium perchlorate in acetonitrile or pyridine in the presence of 2,4,6-trimethylpyridine. Examples of the use of this new group are given in Scheme 19.

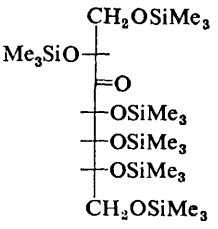
A heavily substituted *O*-(3-aminopropyl)sucrose (containing about seven ether groups per molecule) has been prepared from the corresponding 2-cyanoethyl ether and was used as a bifunctional catalyst for the dedeuteriation of [2-^{2}H]isobutyraldehyde.[174]

Silyl Ethers.—The synthesis and reactions (acidic and basic hydrolyses, removal with tetrabutylammonium fluoride) of a number of t-butyldimethylsilyl ethers of aldose and alditol derivatives have been reported, together with their i.r. and m.s. characteristics.[175]

Further studies (*cf.* Vol. 3, p. 32) on the trimethylsilylation of coriose (D-*altro*-3-heptulose), which exists as the α-furanose form in the crystalline state, have shown that prolonged treatment with trimethylsilyl chloride in pyridine causes the initial product (53) to equilibrate with the ethers (54) and (55).[176]



(53) R^1 = H; R^2 = Me_3Si
(54) R^1 = R^2 = Me_3Si



(55)

Intramolecular Ethers (Anhydro-sugars)

Epoxides.—Several 1,2-diols have been converted into epoxides under mild conditions by treatment with the sulphurane (56). Thus, *trans*-cyclohexane-1,2-diol gave the expected epoxide in 97% yield, whereas the *cis*-diol gave cyclo-

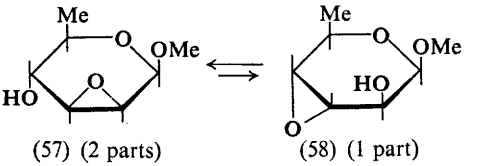
$$Ph_2S[OC(CF_3)_2Ph]_2$$
(56)

hexanone and products other than the epoxide.[177] The reagent may find some application in carbohydrate chemistry. A one-step, high-yielding synthesis of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside has involved the use of an excess of *N*-toluene-

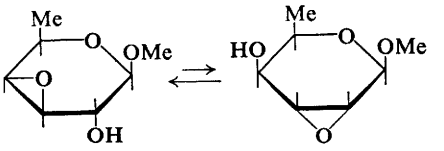
[173] A. Ya. Khorlin, V. A. Nesmeyanov, and S. E. Zurabayan, *Carbohydrate Res.*, 1974, **33**, C1.
[174] J. Hine and S. S. Ulrey, *J. Org. Chem.*, 1974, **39**, 3231.
[175] B. Kraska, A. Klemer, and H. Hagedorn, *Carbohydrate Res.*, 1974, **36**, 398.
[176] T. Okuda, K. Konishi, and S. Saito, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1624.
[177] J. C. Martin, J. A. Franz, and R. J. Arhart, *J. Amer. Chem. Soc.*, 1974, **96**, 4604.

p-sulphonylimidazole with sodium hydride in dry DMF.[178] The use of 1.5 mol of sodium methoxide and this sulphonylating reagent also furnished the intermediate 2-toluene-*p*-sulphonate in high yield.

Buchanan and his colleagues have extended their work on sugar epoxides in a report on the synthesis and properties of 2,3-anhydro-D-mannose and 3,4-anhydro-D-altrose, which were obtained by hydrogenolysis of the corresponding benzyl glycosides.[179] Following mutarotation of crystalline 2,3-anhydro-β-D-mannopyranose, the solution contained the α-pyranose (23%), β-pyranose (7%), α-furanose (65%), and β-furanose (5%) forms at equilibrium, demonstrating that the fused anhydro-ring has a very substantial influence on the behaviour of D-mannose in solution. The same group has also examined the base-catalysed equilibrations of the pairs of 6-deoxy derivatives (57) and (58), and (59) and



(57) (2 parts) (58) (1 part)

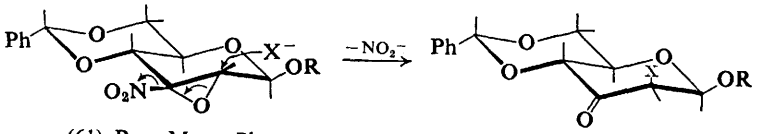


(59) (9 parts) (60) (1 part)

(60).[180] The proportions of the anhydro-sugars at equilibrium were rationalized in terms of the preferred conformations determined by ^{1}H n.m.r. spectroscopy.

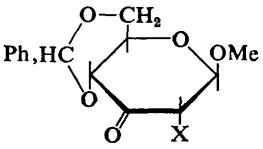
The kinetics of ring-opening by azide ions of a series of methyl 2,3-anhydro-4,6-*O*-benzylidene-α- and -β-D-glycopyranosides have been compared.[181] From the relative rates of the reactions and from the stereochemistries of the products, it was suggested that, in addition to factors already known to influence the ring-opening reactions, the orientation of groups adjacent to the reaction centres is important. In the case of the nitro-oxiran (61), electronic factors direct incoming nucleophiles to position 2, and ring-opening is accompanied by loss of the nitro-group (Scheme 20).[182] In the case of the methyl β-glycoside (61; R = Me), attack by bromide, azide, and deuteride ions afforded the compounds (62)—(64), resulting from isomerization of the initial products of ring-opening. It is perhaps surprising that the bromo-compound (62) is preferred, since the well-known 'α-haloketone effect' should disfavour this epimer. With the phenyl β-glycoside (61; R = Ph), β-elimination of the glycosidic substituent yielded the enones (65).

[178] D. R. Hicks and B. Fraser-Reid, *Synthesis*, 1974, 203.
[179] J. G. Buchanan and D. M. Clode, *J.C.S. Perkin I*, 1974, 388.
[180] S. A. S. Al Janabi, J. G. Buchanan, and A. R. Edgar, *Carbohydrate Res.*, 1974, **35**, 151.
[181] R. D. Guthrie and J. A. Leibmann, *Carbohydrate Res.*, 1974, **33**, 355.
[182] S. Kumazawa, T. Sakakibara, R. Sudoh, and T. Nakagawa, *Angew. Chem. Internat. Edn.*, 1973, **12**, 921.

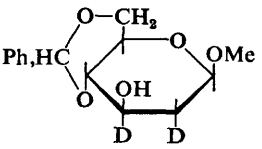


(61) R = Me or Ph

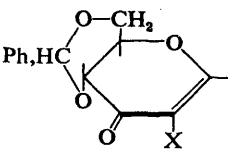
Scheme 20



(62) X = Br
(63) X = N_3

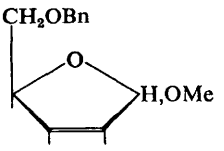


(64)

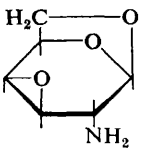


(65) X = Br or N_3

Studies directed at the synthesis of hexosides from unresolved 2-alkoxydihydropyrans and 3,4-anhydrides derived therefrom have been reported,[183] and the compound (66), obtained from L-glutamic acid (see Vol. 5, p. 7), has been used in the preparation of the isomeric methyl 2,3-anhydro-5-*O*-benzyl-D-pentofuranosides, which were subsequently transformed into deoxy, amino, and thio derivatives by standard ring-opening reactions.[184]



(66)



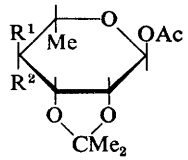
(67)

A 2,3-anhydro derivative obtained from methyl β-maltoside has been converted into the corresponding 3-bromo-3-deoxymaltoside by diequatorial ring-opening with hydrogen bromide.[185]

In the 1,6:3,4-dianhydrohexose series, compound (67) has been prepared and represents the first sugar derivative having an amino-group adjacent and *trans* to an epoxide ring.[186] The aziridine derivative was not obtained when (67) was treated with alkali under conditions that isomerize the hydroxy-analogue. Other epoxides are mentioned in Chapters 6, 8, 14, and 21.

Other Anhydrides.—Treatment of the 4-methanesulphonates (68) and (69) with sodium azide in DMF at 140 °C gave the corresponding 1,4-anhydropyranoses, possessing boat conformations, following initial cleavage of the 1-acetate group.[187] A related compound, 1,4-anhydro-2,3,6-tri-*O*-benzyl-β-D-glucopyranose, yielded a linear 1,4-linked polysaccharide, containing only pyranoid rings

[183] A. Banaszek, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1974, **22**, 79 (*Chem. Abs.*, 1974, **80**, 108 769y).
[184] M. Taniguchi, K. Koga, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2318.
[185] P. L. Durette, L. Hough, and A. C. Richardson, *J.C.S. Perkin I*, 1974, 88.
[186] M. Černý, O. Juláková, and J. Pacák, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1391.
[187] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *J.C.S. Perkin I*, 1973, 2691.

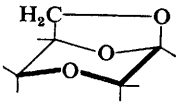


(68) R^1 = OMs; R^2 = H
(69) R^1 = H; R^2 = OMs

involved mainly in β-linkages, when treated with triethyloxonium tetrafluoroborate.[188] Whereas protic-acid treatment of 2,3,6-tri-*O*-benzyl-D-glucose gave the 1,4-anhydride, Lewis acids (PF_5 and SbF_5, *etc.*) catalysed the polymerization of this product to polysaccharides containing furanoid and pyranoid rings joined mainly by α-linkages.[188a]

Acetoxonium ions formed from several 1,6-anhydrohexose 2,3,4-triacetates are covered in detail in Chapter 6. However, it is notable that derivatives of 1,6-anhydrides were among the products formed when the penta-acetates of D-glucopyranose, D-mannopyranose, and D-altropyranose and the corresponding acetylated methyl glycosides reacted with anhydrous hydrogen fluoride.[189] A synthesis of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose from phenyl β-D-galactopyranoside tetra-acetate has been described.[190]

Hydrogenolysis of the 3,6,8-trioxabicyclo[3,2,1]octane (70) (a 1,6-anhydrohexose analogue) with lithium aluminium hydride and aluminium chloride



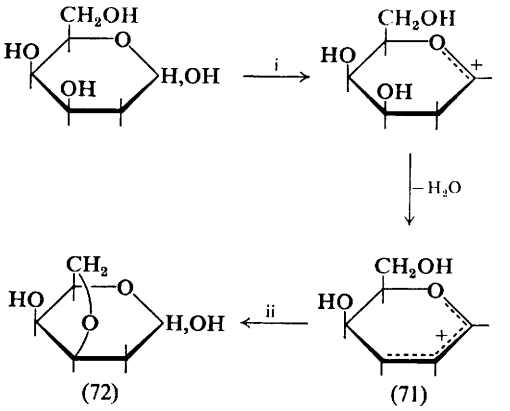
(70)

resulted in cleavage of the five-membered ring, rather than the six-membered ring, to furnish 1,4-dioxans bearing a hydroxymethyl group.[191]

2,3,4-Tri-*O*-methyl-D-xylose dimethyl acetal underwent ring-closure and specific demethylation on toluene-*p*-sulphonylation to give the 2,5-anhydro derivative.[192] A development of this study is dealt with in Chapter 11.

Incubation of 2-deoxy-D-*lyxo*-hexose with β-galactosidase gave the 3,6-anhydro derivative (72) (*ca.* 2%) by way of the allylic carbonium ion (71) (Scheme 21).[193] 2-Amino-3,6-anhydro-2-deoxy-D-glucose has been prepared by way of the 6-toluene-*p*-sulphonate, and it was reduced to give 2-acetamido-3,6-anhydro-2-deoxy-D-glucitol after *N*-acetylation;[194] a derivative of 3,6-anhydromaltose has

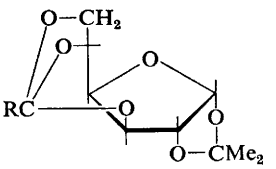
[188] F. Micheel and O.-E. Brodde, *Annalen*, 1974, 702.
[188a] F. Micheel, O.-E. Brodde, and K. Reinking, *Annalen*, 1974, 124.
[189] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1973, **27**, 2701.
[190] M. Sozmen, *Comm. Fac. Sci. Univ. Ankara, Ser. B*, 1972, **19**, 99 (*Chem. Abs.*, 1974, **80**, 96 236g).
[191] J. Gelas and S. Veyssieres-Rambaud, *Carbohydrate Res.*, 1974, **37**, 293.
[192] T. Van Es, *Carbohydrate Res.*, 1974, **37**, 373.
[193] J. Lehmann and E. Schröter, *Carbohydrate Res.*, 1974, **36**, 303.
[194] E. Walker, P. Roussel, R. W. Jeanloz, and V. N. Reinhold, *Carbohydrate Res.*, 1974, **35**, 270.



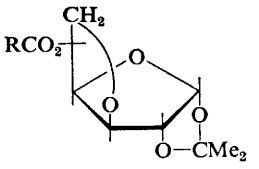
Reagents: i, β-galactosidase; ii, H_2O

Scheme 21

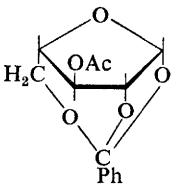
been similarly prepared.[185] In the furanose series, it has been shown that the 3,5,6-orthoester (73) is converted with acid into the 3,6-anhydride (74).[194a] In related fashion, treatment of the orthobenzoate (75) with mercuric bromide in acetonitrile gave the intramolecular (76) and intermolecular (77) anhydrides.[195]



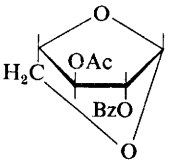
(73) R = H or Me



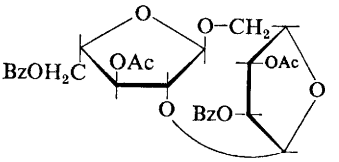
(74) R = H or Me



(75)



(76)

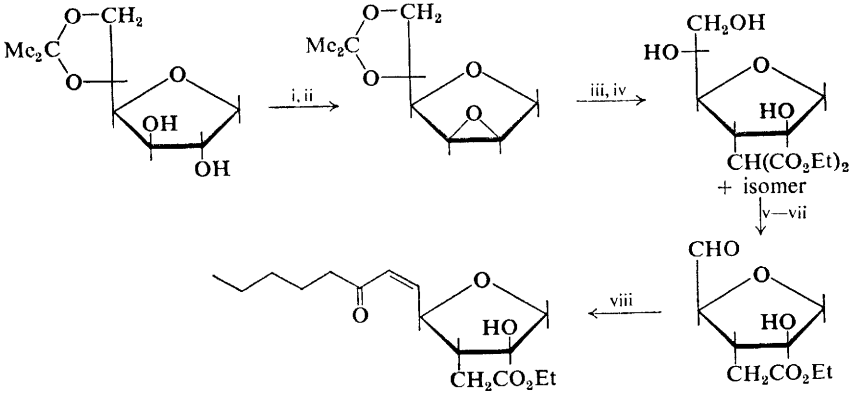


(77)

A related 1,5′:1′,5-dianhydride resulted from treatment of 6-deoxy-D-allose with benzaldehyde and zinc chloride.[196]

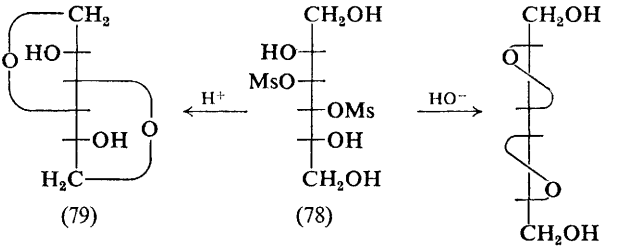
A chiral precursor for the 11-oxaprostaglandins has been synthesized from a derivative of 1,4-anhydro-D-glucitol (Scheme 22).[197]

[194a] P. Köll and H. Meyborg, *Tetrahedron Letters*, 1974, 4499.
[195] A. F. Bochkov, I. V. Obruchnikov, V. N. Chernetsky, and N. K. Kochetkov, *Carbohydrate Res.*, 1974, **36**, 191.
[196] R. G. S. Ritchie, J. F. Stoddart, D. M. Vyas, and W. A. Szarek, *Carbohydrate Res.*, 1974, **32**, 279.
[197] S. Hanessian, P. Dextrase, A. Fougerousse, and Y. Guindon, *Tetrahedron Letters*, 1974, 3983.



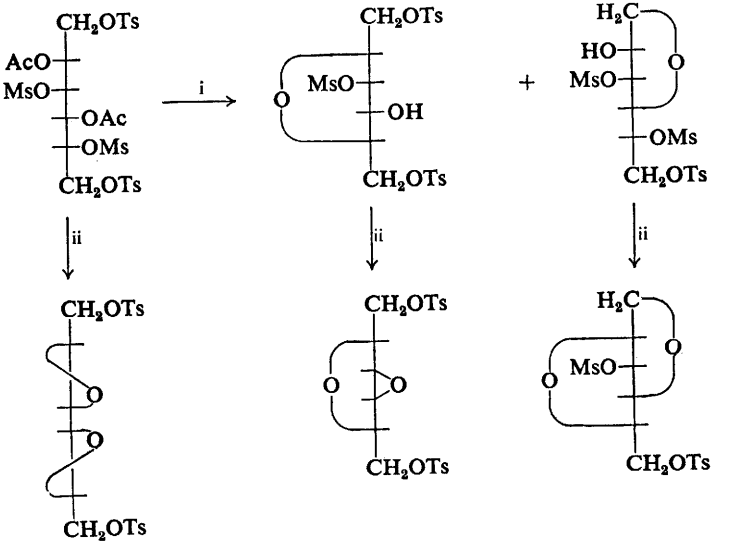
Reagents: i, MsCl–py; ii, NaOMe; iii, $NaCH(CO_2Et)_2$; iv, H_3O^+; v, NaOH; vi, EtOH–H^+; vii, $NaIO_4$; viii, $(MeO)_2POCH_2COC_5H_{11}$

Scheme 22



(79) (78) (80)

Scheme 23



Reagents: i, H^+; ii, NaOMe

Scheme 24

Parallel studies have been carried out on the formation of anhydro-rings on acid and base treatments of D-mannitol sulphonates. The results indicated that epoxides are generally formed under basic conditions, whereas acid promotes the formation of five-membered anhydro-rings. Thus, 3,4-di-*O*-methylsulphonyl-D-mannitol (78) afforded 1,4:3,6-dianhydro-D-iditol (79) under acid conditions, whereas the 2,3:4,5-dianhydride (80) was obtained with base (Scheme 23). Hydrolysis of the dianhydride (80) with acid gave only a small proportion of (79), implying that the bis(oxiran) is not formed from (78) with acid.[198] Scheme 24 also illustrates these generalizations and shows that five-membered rings were formed under basic conditions if stereochemical factors precluded the formation of epoxides.[199]

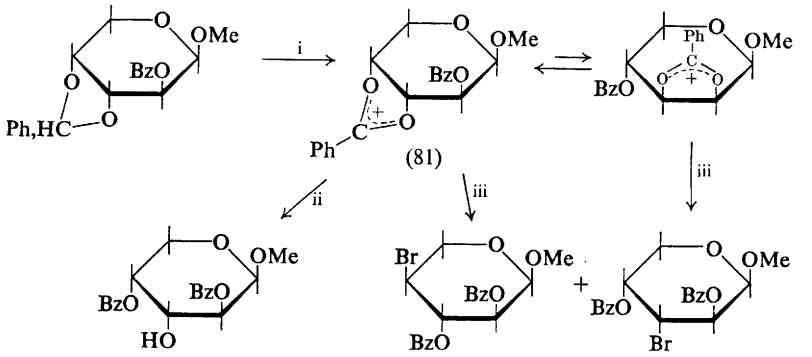
[198] D. R. Hicks and B. Fraser-Reid, *Canad. J. Chem.*, 1974, **52**, 3367.
[199] J. Kuszmann and P. Sohár, *Carbohydrate Res.*, 1974, **35**, 97.

5
Acetals

Reactions and Properties of Acetals

The ring-opening of cyclic acetals has been commented upon in a number of papers. It has been shown that borane in THF effects the reductive cleavage of acetals with the formation of hydroxyethers; the mechanism appeared to resemble that of reduction with mixtures of lithium aluminium hydride and aluminium chloride.[200] Benzylidene acetals in particular gave high yields of monobenzylated diols. The reductive opening of 4,6-*O*-benzylidene rings fused to D-glucopyranosides afforded both 4- and 6-*O*-benzyl ethers, the ratios of which depended on the substituent at C-3.[154] *NN*-Dibromobenzenesulphonamide, like *N*-bromosuccinimide, can be used to cleave benzylidene acetals to monobromo-monobenzoates,[201] whereas electrochemical methods can be used to remove benzylidene groups with formation of the parent diol.[202]

The use of trityl tetrafluoroborate has afforded another means of generating cyclic acyloxonium ions, and Pedersen has employed this reagent to generate the benzoxonium ion (81) shown in Scheme 25, which also illustrates some of the reactions of this and the isomeric ion.[203] Methyl 4-*O*-benzoyl-2,3-*O*-benzylidene-α-D-lyxopyranoside and methyl 5-*O*-benzoyl-2,3-*O*-benzylidene-β-D-ribofuranoside gave similar results. The selective reactivity of the five-membered acetal

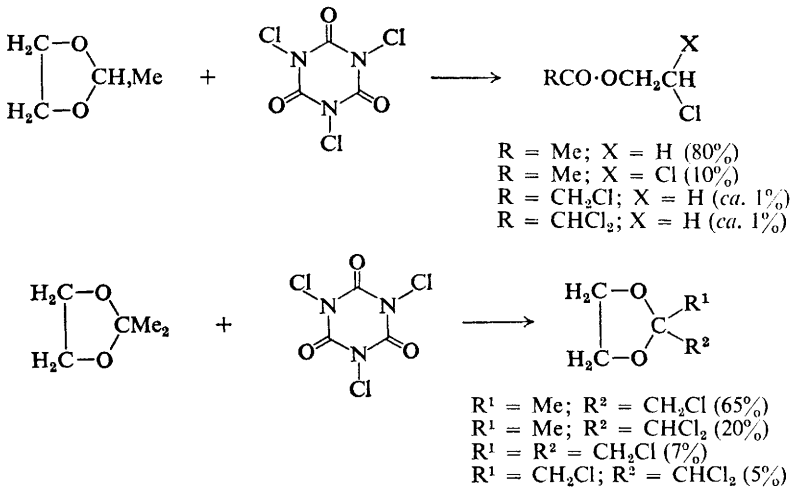


Scheme 25

[200] B. Fleming and H. I. Bolker, *Canad. J. Chem.*, 1974, **52**, 888.
[201] Y. Kamiya and S. Takemura, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 201.
[202] V. G. Mairanovskii, N. F. Loginova, A. M. Ponomarev, and A. Ya. Veinberg, *Electrokhimiya*, 1974, **10**, 172 (*Chem. Abs.*, 1974, **80**, 108 770s).
[203] S. Jacobsen and C. Pedersen, *Acta Chem. Scand.* (*B*), 1974, **28**, 866.

ring in methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside with trityl tetrafluoroborate furnished the D-mannopyranoside 2-benzoate (with water) and methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-bromo-3-deoxy-α-D-altropyranoside (with tetraethylammonium bromide).[204] However, this type of reactivity was not shown by 1,2-*O*-benzylidene acetals fused to either pyranose or furanose rings.

The reactions of acetals with the powerful chlorinating agent 1,3,5-trichloro-1,3,5-triazine-2,4,6-trione are illustrated in Scheme 26.[205]



Scheme 26

A development of Horton's interest in the conformations of acyclic derivatives in solution has led to a study of unsubstituted D-aldopentose diethyl and diphenyl dithioacetals. The usual planar and slightly distorted conformations were identified, and the conformations assumed showed little variation with changes of solvent.[206]

Synthesis

Amberlyst, a styrene–divinylbenzene sulphonic acid resin, has been recommended for use in the preparation of acetals,[207] and the use of acid resins in the presence of anhydrous calcium sulphate has also been advocated.[208]

Acetals Derived from Carbohydrate Carbonyl Groups.—The dimethyl acetals of D-galacturono-6,3-lactone and methyl D-galacturonate have been identified as the kinetic products of methanolysis of D-galacturonic acid, and their combined maximum concentration was estimated to be *ca.* 10%.[55] The diethyl dithioacetal

[204] S. Jacobsen and C. Pedersen, *Acta Chem. Scand.* (*B*), 1974, **28**, 1024.
[205] J. Gelas and D. Petrequin, *Carbohydrate Res.*, 1974, **36**, 227.
[206] D. Horton and J. D. Wander, *J. Org. Chem.*, 1974, **39**, 1859.
[207] S. A. Patwardhan and S. Dev, *Synthesis*, 1974, 348.
[208] V. I. Stenberg and D. A. Kubik, *J. Org. Chem.*, 1974, **39**, 2815.

and the dimethyl acetal of *N*-acetylmuramic acid (as the 5,6-*O*-isopropylidene derivatives) have been reported, together with several related compounds.[209] Dimethyl acetals of pyranosiduloses are referred to in Chapter 16.

Acetals Derived from Carbohydrate Hydroxy-groups.—The use of ethyl isopropenyl ether in DMF in the presence of an acid catalyst has been recommended for the synthesis of certain *O*-isopropylidene derivatives.[210] A near-quantitative yield of 4,6-*O*-isopropylidene-D-glucopyranose, rather than the usual 1,2:5,6-diacetal, was obtained from D-glucose, and it was suggested that the reagent reacts most readily with primary hydroxy-groups, giving rise to cyclic acetals that do not rearrange. Methyl α-D-glucopyranoside furnished the 4,6-acetal and D-mannitol a mixture of 1,2:5,6-di- and 1,2:3,4:5,6-tri-*O*-isopropylidene derivatives.

1,2:5,6-Di-*O*-isopropylidene-α-D-galactofuranose is formed in only small proportions by the usual methods of acetonation; a modification, involving dissolution of the hexose in hot DMF prior to treatment with acetone in the presence of copper(II) sulphate, has been reported to give this diacetal directly in 20% yield.[211] Mass spectrometry has been used to characterize the 4,6- and 3,5-*O*-furylidene derivatives of D-glucose and D-xylose, respectively.[211a]

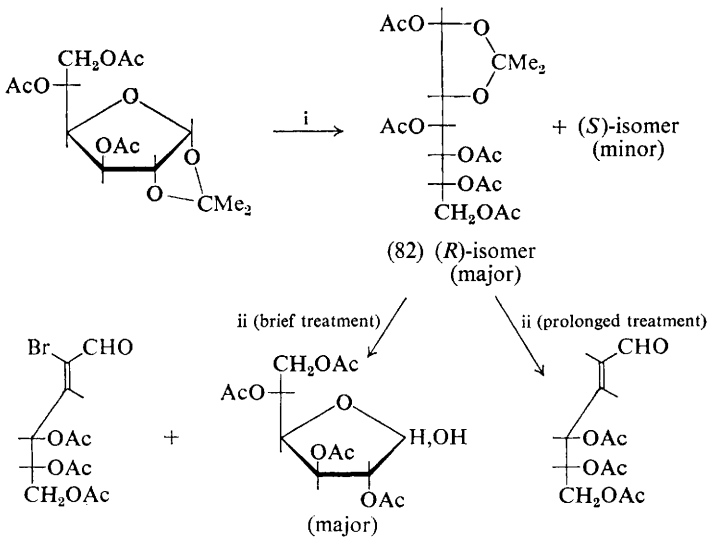
A number of features of the synthesis of cyclic acetals of glycosides have been reported. Improved methods for the synthesis of *o*-nitrobenzylidene derivatives of aldopyranosides, and the separation of a number of them into *endo*- and *exo*-forms, have been described,[212] and diastereoisomers of methyl 4,6-*O*-(1-benzylethylidene)-α-D-glucopyranoside have been prepared using 2,2-dimethoxy-1-phenylpropane.[213] Other 4,6-acetals of methyl α-D-glucopyranoside have been obtained by reaction with anisaldehyde, vanillin, veratrylaldehyde, and syringaldehyde.[214] The base-catalysed procedure with benzylidene bromide in pyridine has been used to prepare 4,6-*O*-benzylidenesucrose.[215] Structural studies on a *Klebsiella* capsular polysaccharide have indicated the presence of three contiguous L-rhamnosyl residues, the central residue of which appears to carry a 1-carboxyethylidene acetal bridging the *trans*-diol grouping at O-3 and O-4.[216]

Contrary to earlier evidence, it has been shown that ring-contraction does not occur on treatment of methyl β-D-ribopyranoside with acetone in the presence of an acid catalyst, but that the 2,3- and 3,4-acetals are formed in the proportion 1 : 2.[217] An interesting use of the solid-phase technique has shown that specifically substituted acetals can be obtained using suitable resins.[218] A polymer supporting

[209] A. Veyrieres, *Carbohydrate Res.*, 1974, **33**, 203.
[210] M. L. Wolfrom, A. B. Diwadkar, J. Gelas, and D. Horton, *Carbohydrate Res.*, 1974, **35**, 87.
[211] S. Morgenlie, *Acta Chem. Scand.*, 1973, **27**, 3609.
[211a] V. Kováčik, P. Kováč, M. Kosik, and V. Demianova, *Chem. Zvesti*, 1974, **28**, 270 (*Chem. Abs.*, 1974, **81**, 49 939z).
[212] P. M. Collins and N. N. Oparaeche, *Carbohydrate Res.*, 1974, **33**, 35.
[213] D. Joniak, B. Košiková, and R. Palovčík, *Carbohydrate Res.*, 1974, **36**, 181.
[214] D. Joniak and B. Košiková, *Chem. Zvesti*, 1974, **28**, 110 (*Chem. Abs.*, 1974, **80**, 146 460g).
[215] R. Khan, *Carbohydrate Res.*, 1974, **32**, 375.
[216] Y.-M. Choy and G. G. S. Dutton, *Canad. J. Chem.*, 1974, **52**, 684.
[217] N. A. Hughes and C. D. Maycock, *Carbohydrate Res.*, 1974, **35**, 247.
[218] S. Hanessian, T. Ogawa, Y. Guindon, J. L. Kamennof, and R. Roy, *Carbohydrate Res.*, 1974, **38**, C15.

benzaldehyde residues condensed with the 2,3-diol grouping of methyl β-D-ribofuranoside to give an immobilized *O*-benzylidene derivative having the primary hydroxy-group free for substitution. Phosphorylation of nucleosides at O-5′ can likewise be effected using functionalized polymers. Methanesulphonylation of polymer-bound methyl α-D-glucopyranoside took place selectively at O-2, whereafter careful acid hydrolysis released methyl 2-*O*-methylsulphonyl-α-D-glucopyranoside.

The unusual acyclic compound (82) has been obtained by the action of acetic anhydride and zinc chloride on 3,5,6-tri-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose, and Scheme 27 outlines its reactions with hydrogen bromide in



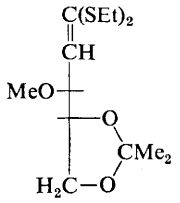
Reagents: i, Ac_2O–$ZnCl_2$; ii, HBr–HOAc

Scheme 27

acetic acid; possible mechanisms for the formation of these products were also discussed.[219] Treatment of D-xylose diethyl dithioacetal with acetone in the presence of phosphorus pentoxide has been shown to yield the 2,3:4,5-di-*O*-isopropylidene derivative and not the 2,4:3,5-isomer claimed previously.[220] Attempted methylation of the 2,3:4,5-diacetal using dimethyl sulphate and potassium hydroxide gave the unsaturated derivative (83). The reaction of 2,3,4,5-tetra-*O*-methyl-D-xylose diethyl dithioacetal (84) with methyl iodide and silver oxide afforded a large number of products, possibly including the dimethyl acetal (85) and the aldonate methyl ester (86) (see Scheme 28).

The use of 2,2-dimethoxypropane has been recommended for the preparation of 1,2:5,6-di-*O*-isopropylidene-D-mannitol,[221] and the acid-catalysed reaction of

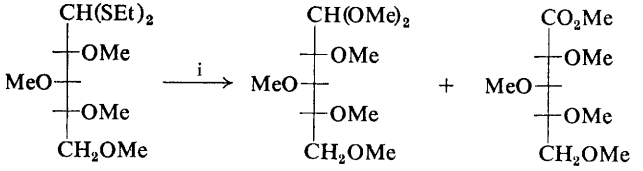
[219] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1974, **28**, 853.
[220] T. Van Es, *Carbohydrate Res.*, 1974, **32**, 370.
[221] G. Kohan and G. Just, *Synthesis*, 1974, 192.



(83)

galactitol with butyraldehyde yielded the 1,3- and 2,3-mono- and 1,3:4,6-di-acetals.[222]

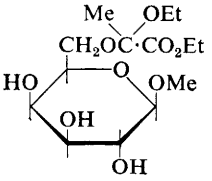
Only one report has described acetals derived by reaction with a single hydroxy-group of carbohydrates. Thus, an acetal-exchange reaction between methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside and ethyl pyruvate diethyl acetal gave diastereoisomeric acetals, which were transformed into the 6-acetal (87) following debenzoylation.[223] A similar procedure was used to obtain the isomeric 4-acetal.



(84) (85) (86)

Reagents: i, MeI–Ag_2O

Scheme 28



(87)

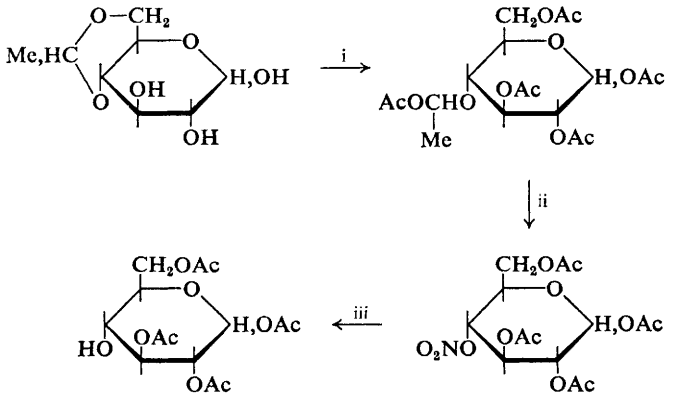
[222] T. G. Bonner, E. J. Bourne, D. Lewis, and L. Yüceer, *Carbohydrate Res.*, 1974, **33**, 1.
[223] I. E. Valashek, M. K. Shakhova, V. A. Minaev, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1974, **44**, 1161.

6
Esters

Carboxylic Esters

Considerations leading to the maximal yield of each monosubstituted derivative in the acylation or alkylation of diols have been described [149] (see also Chapter 4).

Unimolar acetylation of methyl 6-*O*-trityl-α-D-glucopyranoside gave preferentially the 4-acetate, which was also formed from the 2- and 3-acetates under mild alkaline conditions.[224] Partial acetylation of methyl 3-acetamido-3,6-dideoxy-β-D-gluco- and -manno-pyranosides has shown the hydroxy-group at C-2 to be more reactive in each case,[225] and selective acetylation (acetic anhydride–sodium acetate) of a series of benzyl hexopyranosides gave the following results: β-D-*galacto* 2,3,6- (25%), 2,4,6- (9%), and 3,4,6-triacetates (3%); β-D-*gluco* 2,4,6-triacetate (66%); α-D-*manno* 2,3,6-tri- (25%) and 2,3,4,6-tetra-acetates (65%).[226] Syntheses of 1,2,3,6-tetra-*O*-acetyl-α- and -β-D-glucopyranoses are illustrated in Scheme 29; their use in Koenigs–Knorr reactions led to acetyl

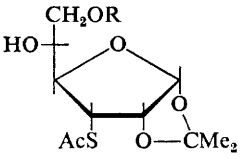


Reagents: i, Ac_2O–H_2SO_4; ii, Ac_2O–HNO_3; iii, AcOH–Zn–Fe

Scheme 29

migration from O-6 to O-4, resulting in the formation of (1 → 6)-linked oligosaccharides.[73] Silica-gel chromatography of the thioacetates (88) and (89) led to acetyl migration from S-3 to O-5 and -6, respectively, whereas the corresponding

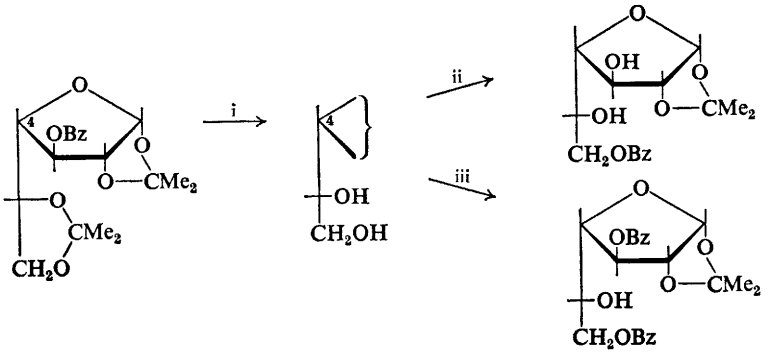
[224] C. G. Casinovi, M. Framondino, G. Randazzo, and F. Siani, *Carbohydrate Res.*, 1974, **36**, 67.
[225] K. Čapek, J. Staněk, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2694.
[226] E. E. Lee, A. Bruzzi, E. O'Brien, and P. S. O'Colla, *Carbohydrate Res.*, 1974, **35**, 103.



(88) R = Bz
(89) R = H

O-acetates were stable under comparable conditions.[227] Both 1′,2,3,3′,4′,6,6′- and 1′,2,3,3′,4,6,6′-hepta-acetates of sucrose have been identified among the mixture of products obtained on selective deacetylation of the octa-acetate with alumina, sucrose 1′,2,3,3′,4,4′,6′-hepta-acetate having been identified previously.[228]

Syntheses of the 3- and 6-mono- and 3,6-di-benzoates of 1,2-*O*-isopropylidene-α-D-galactofuranose are shown in Scheme 30.[229] Benzoylation of methyl 2-*O*-



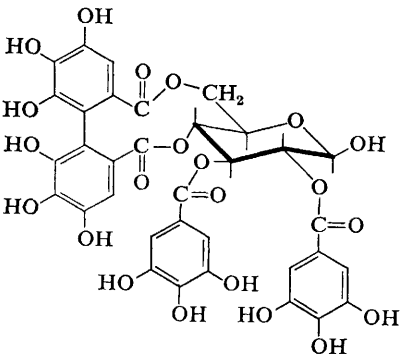
Reagents: i, H_3O^+; ii, NaOH; iii, *N*-benzoylimidazole

Scheme 30

methyl-β-L-arabinopyranoside occurred preferentially at the equatorial hydroxy-group at C-3, whereafter methylation and saponification furnished the 2,4-di-*O*-methyl ether.[230] Partial benzoylation of benzyl 6-*O*-methyl-β-D-galactopyranoside gave a 2,3-dibenzoate, which was converted into 6-*O*-methyl-D-galactose 4-sulphate by standard procedures.[231] The relative reactivities of the hydroxy-groups of *N*-acetyl-*N*-aryl-β-D-xylopyranosylamines towards benzoylation were shown to be HO-3 ≈ HO-4 > HO-2.[232] Dimolar benzoylation of methyl β-lactoside with benzoyl chloride in pyridine afforded the 3′,6′-di- (31%), 3,6,6′-tri- (31%), 2,3′,6,6′-tetra- (22%), 2,2′,3′,6,6′-penta- (9%), and 2,3′,4′,6,6′-penta-benzoates (9%), indicating that the order of reactivity of the hydroxy-groups is 6′ > 3′ > 6 > 2 > 2′ ≈ 4′ > 3.[233]

[227] R. L. Whistler, A. K. M. Anisuzzaman, and J. C. Kim, *Carbohydrate Res.*, 1973, **31**, 237.
[228] J. M. Ballard, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1974, **34**, 184.
[229] C. L. Brewer, S. David, and A. Veyrieres, *Carbohydrate Res.*, 1974, **36**, 188.
[230] P. Kováč and R. Palovčík, *Carbohydrate Res.*, 1974, **36**, 379.
[231] J. F. Batey and J. R. Turvey, *Carbohydrate Res.*, 1974, **38**, 316.
[232] Z. Smiatacz, *Carbohydrate Res.*, 1974, **38**, 117.
[233] R. S. Bhatt, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1974, **32**, C4.

A number of *N*-benzyloxycarbonyldipeptidyl esters of 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranoses have been synthesized; in certain instances, hydrogenolysis of these derivatives led to intramolecular aminolysis to form diketopiperazines with cleavage of the glycosidic ester bond.[234] 3-Acylpropionyl derivatives of carbohydrates were readily cleaved by hydrazine hydrate in pyridine–acetic acid, thereby offering an alternative method for the protection of hydroxy-groups.[235] New monoesters of indole-3-acetic acid and D-glucose, in which the hexose is esterified at O-2, -3, -4, and -6, have been isolated from sweet-corn kernels of *Zea mays*,[236] and 1-*O*-(indole-3-acetyl)-β-D-glucopyranose labelled with ^{14}C in the carboxy-group has been synthesized by standard methods.[236a] *N*-(Tri-*O*-methylgalloyl)- and *N*-(tri-*O*-benzylgalloyl)-imidazoles have been used to prepare fully [237] and partially [238] acylated derivatives of methyl α-D-glucopyranoside and its 6-*O*-trityl ether; thus, partial acylation gave the 6-mono-, 2,6-di-, and 2,3,6-tri-substituted derivatives of the former and the 2-mono- and 2,3-di-substituted derivatives of the latter. Kollinin, a hydrolysable tannide from geranium leaves, has been identified as (90).[239] The fusion of



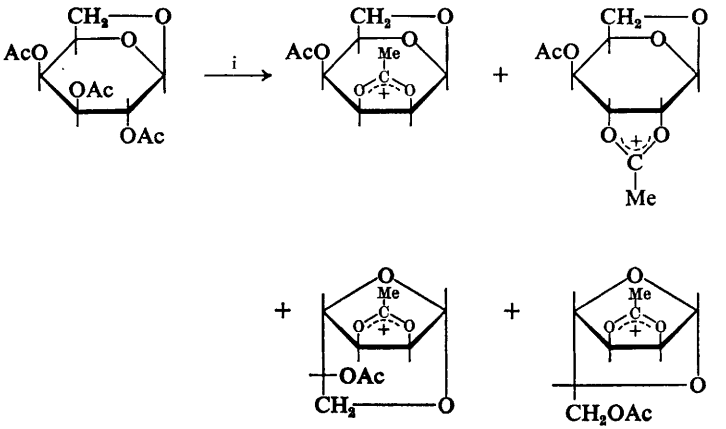
(90)

D-glucose and citric acid has furnished two monoesters, each with a citrate group attached to the primary hydroxy-group of the hexose, as the principal products.[240] The 3-*O*-benzoylformate or pyruvate esters of 1,2-*O*-cyclohexylidene-α-D(L)-xylofuranose, attached by an ether linkage through O-5 to a polymer support, have been used in the asymmetric synthesis of α-hydroxy-acids by addition of Grignard reagents; good optical yields (*ca.* 60%) were obtained in most cases.[241]

[234] D. Keglević and Š. Valenteković, *Carbohydrate Res.*, 1974, **38**, 133.
[235] R. D. Guthrie, T. J. Lucas, and R. Khan, *Carbohydrate Res.*, 1974, **33**, 391.
[236] A. Ehmann, *Carbohydrate Res.*, 1974, **34**, 99.
[236a] F. Sirokman and E. Koves, *Acta Phys. Chem.*, 1974, **20**, 121.
[237] L. Birkofer and K. Idel, *Annalen*, 1974, 1.
[238] L. Birkofer and K. Idel, *Annalen*, 1974, 4.
[239] T. N. Bikbulatova and T. K. Chumbalov, *Fenol'nye Soedinenii Ikh. Fiziol. Svoiston, Mater. Vseo. Simp. Fenol'nym Soedinenii, 2nd*, 1971 (Pub. 1973), p. 138.
[240] H. G. Maier and H. Ochs, *Chem. Mikrobiol. Technol. Lebensm.*, 1973, **2**, 79 (*Chem. Abs.*, 1974, **80**, 83 433u).
[241] M. Kawana and S. Emoto, *Bull. Chem. Soc. Japan*, 1974, **47**, 160.

Acyloxonium Ions and Orthoesters

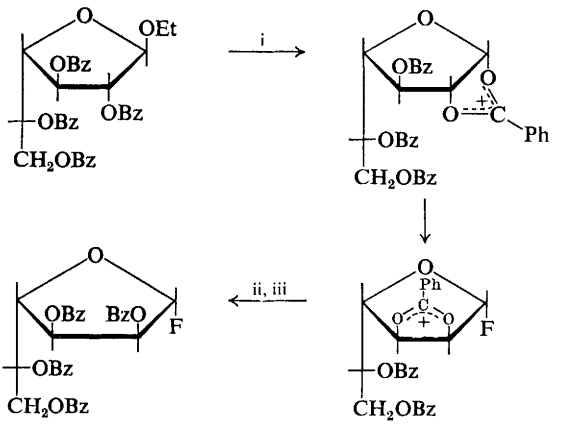
Durette and Paulsen have examined the acetoxonium ions produced from a series of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-hexopyranoses having the D-*gluco*, D-*talo*,[242] D-*galacto*,[243] D-*allo*, D-*altro*, and D-*manno* configurations.[244] As well



Reagent: i, CF_3SO_3H

Scheme 31

as epimerizations resulting from acetoxonium-ion migration, ring-contractions also occurred; *e.g.*, the acetoxonium ions arising from 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose are shown in Scheme 31. Benzoxonium ions were

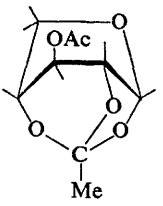


Reagents: i, HF; ii, H_2O; iii, BzCl–py

Scheme 32

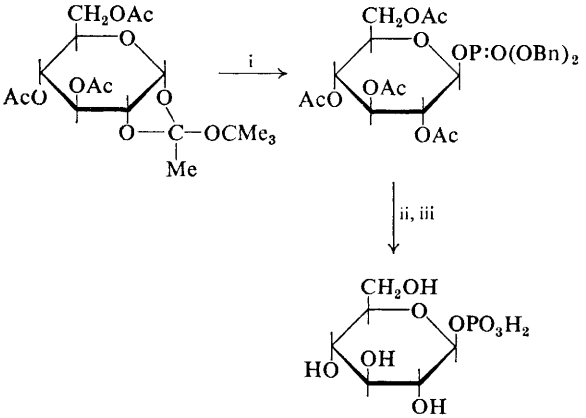
[242] P. L. Durette and H. Paulsen, *Carbohydrate Res.*, 1974, **35**, 221.
[243] P. L. Durette and H. Paulsen, *Chem. Ber.*, 1974, **107**, 937.
[244] P. L. Durette and H. Paulsen, *Chem. Ber.*, 1974, **107**, 951.

utilized in a synthesis of 2,3,5,6-tetra-*O*-benzoyl-α-D-talofuranosyl fluoride (Scheme 32).[245] Benzoyl borofluorate initiated the polymerization of the D-xylose orthoester (91) to yield a linear polysaccharide containing mainly β-(1 → 4)-linkages and occasional β-(1 → 2)- and β-(1 → 3)-linkages.[246] A new route to



(91)

β-D-glycopyranosyl phosphates, by way of alkyl orthoesters, has been described (Scheme 33).[247] A number of disadvantages associated with earlier methods (M. S. Newman and C. H. Chen, *J. Amer. Chem. Soc.*, 1972, **94**, 2149; *ibid.*, 1973, **95**, 278) for converting the orthoacetates of 1,2-diols into the acetates of chloro-



Reagents: i, $HOP{:}O(OBn)_2$; ii, H_2–Pd; iii, NaOMe

Scheme 33

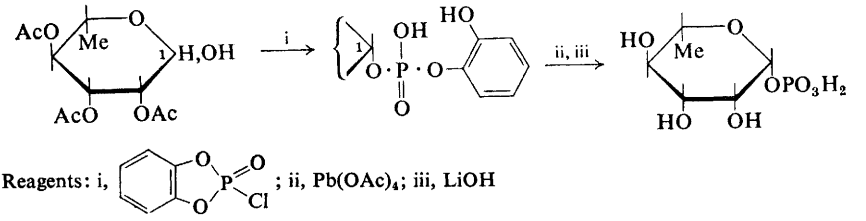
hydrins have been overcome by heating the orthoesters in dichloromethane with an excess of trimethylsilyl chloride.[248]

References to the synthesis of anhydro-sugars from orthoesters and from either peracetylated monosaccharides or glycosides *via* acyloxonium ions are contained in Chapter 4.

[245] K. Bock, C. Pedersen, and L. Wiebe, *Acta Chem. Scand.*, 1973, **27**, 3586.
[246] A. F. Bochkov, I. V. Obruchnikov, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1974, **44**, 1197.
[247] L. V. Volkova, L. L. Danilov, and R. P. Evstigneeva, *Carbohydrate Res.*, 1974, **32**, 165.
[248] M. S. Newman and D. R. Olson, *J. Org. Chem.*, 1973, **38**, 4203.

Phosphates

D-[3-^{2}H]Glyceric acid 2,3-diphosphate has been obtained (following literature procedures) from D-[1,6-$^2H_2$]mannitol[249] (see also Chapter 27). α-D-Glucopyranose 1-phosphate has been reported to have a second dissociation constant (pK_{a_2} 6.46) higher than that of the corresponding β-anomer (pK_{a_2} 6.24).[250] 2-Deoxy-α-D-*arabino*- and -*lyxo*-hexopyranose 1-phosphates have been prepared from the corresponding tetra-acetates by standard routes,[251] and an improved synthesis of β-L-rhamnopyranose 1-phosphate is shown in Scheme 34;[252] *o*-phenylene

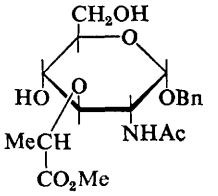


Reagents: i, ; ii, $Pb(OAc)_4$; iii, LiOH

Scheme 34

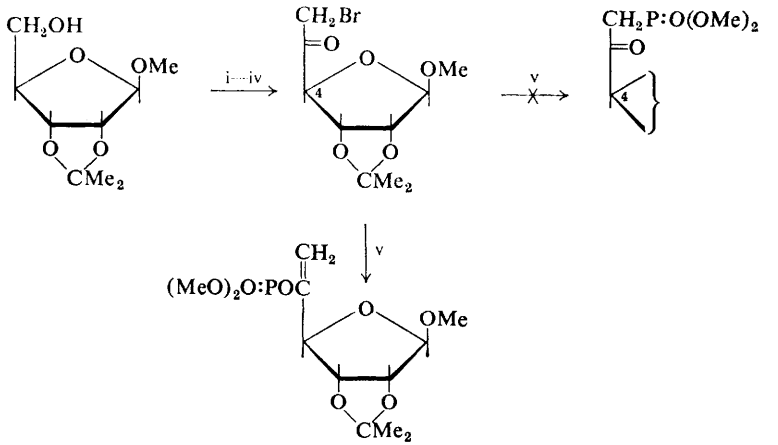
phosphorochloridate has also been used with benzyl 2-acetamido-3-*O*-acetyl-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside to give the 4-phosphate.[253] 3-Deoxy-3-fluoro-D-glucose has been converted into the 1- and 6-phosphates by standard methods,[254] and L-arabinose 5-phosphate[255] and D-*glycero*-L-*manno*-heptose 7-phosphate[256] have been synthesized by way of the 5- and 7-*O*-trityl ethers, respectively. (±)-*myo*-Inositol 1-phosphate has been synthesized *via* phosphorylation of (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol.[257] Phosphate esters have also been obtained by selective phosphorylation; thus, the 6-phosphates of methyl D-galacto-, D-gluco-, and D-manno-pyranosides were obtained using phosphorus oxychloride in trimethyl phosphate.[258] Primary hydroxy-groups were selectively phosphorylated in the presence of secondary hydroxy-groups using *N*-alkylpyridinium salts of five-membered, cyclic acylphosphates.[258a] Muramic acid 6-phosphate has been prepared by way of treatment of the partially protected derivative (92) with diphenyl phosphorochloridate.[259] 3-*O*-Acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose reacted with partially hydrolysed phosphorus oxychloride in pyridine to give the 6-phosphate (isolated as the barium salt); treatment of the barium salt with sodium methoxide or with dilute sulphuric acid gave two cyclic phosphates, presumably the 3,6- and 5,6-cyclic

[249] B. Erbing, P. J. Garegg, and B. Lindberg, *Acta Chem. Scand.* (*B*), 1974, **28**, 1105.
[250] E. J. Behrman, *Carbohydrate Res.*, 1974, **36**, 231.
[251] S. Kucar, J. Zamocky, and S. Bauer, *Chem. Zvesti*, 1974, **28**, 115 (*Chem. Abs.*, 1974, **81**, 4153x).
[252] V. N. Shibaev, Yu. Yu. Kusov, V. A. Petrenko, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1852.
[253] D. R. Bundle and H. J. Jennings, *Canad. J. Biochem.*, 1974, **52**, 723.
[254] J. A. Wright and N. F. Taylor, *Carbohydrate Res.*, 1974, **32**, 366.
[255] M. M. A. Abd El-Rahman and U. Hornemann, *Carbohydrate Res.*, 1974, **38**, 355.
[256] P. Szabo, *J.C.S. Perkin I*, 1974, 920.
[257] D. E. Kiely, G. J. Abruscato, and V. Baburao, *Carbohydrate Res.*, 1974, **34**, 307.
[258] K. Schutzner, *Cesk. Farm.*, 1974, **23**, 34 (*Chem. Abs.*, 1974, **81**, 13 715j).
[258a] F. Ramirez, P. Stern, S. Glaser, I. Ugi, and P. Lemmen, *Phosphorus*, 1973, **3**, 165.
[259] S. Hase and V. Matsushima, *Bull. Chem. Soc. Japan*, 1974, **47**, 1190.



(92)

esters.[260] A method for extracting D-fructose 1,6-diphosphate from yeasts has been reported;[261] the DL-form of this diphosphate was obtained by self-condensation of DL-glyceraldehyde 3-phosphate.[24] An attempt to prepare an isosteric phosphonate analogue of D-ribose 5-phosphate furnished instead a 5-*C*-methylene 5-phosphate (see Scheme 35), the intermediate bromo-compound reacting by the Perkow, rather than the Arbusov, route.[262]



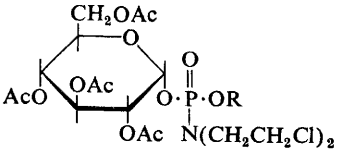
Reagents: i, $KMnO_4$; ii, $SOCl_2$; iii, CH_2N_2; iv, HBr; v, $(MeO)_3P$

Scheme 35

A series of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose 1-amidophosphates (93) have been prepared,[263] and a chemical synthesis of P^1-(2-acetamido-2-deoxy-α-D-glucopyranose-1)-P^2-dolichyl pyrophosphate has been reported.[264]

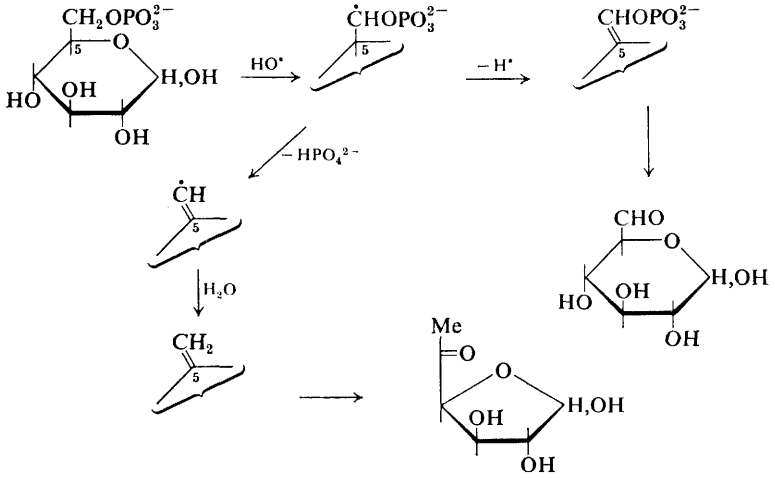
The radiation-induced dephosphorylation of sugar phosphates in aqueous solution has been examined, and the mechanism illustrated with D-glucopyranose 6-phosphate (Scheme 36) was suggested for the process.[265] Complexes of

[260] H. Suzuki and E. Hirayama, *Waseda Daigaku Rikogaku Kenkyusho Hokoku*, 1972, 48 (*Chem. Abs.*, 1974, **80**, 60 107b).
[261] M. Leisola and M. Linko, *Acta Chem. Scand.* (*B*), 1974, **28**, 555.
[262] A. Hampton, F. Perini, and P. J. Harper, *Carbohydrate Res.*, 1974, **37**, 359.
[263] V. M. Ovrutskii, I. I. Kuz'menko, and L. D. Protsenko, *Zhur. obshchei Khim.*, 1974, **44**, 1176.
[264] C. D. Warren and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **37**, 252.
[265] N. K. Kochetkov, L. I. Kudrjashov, M. A. Chlenov, and L. P. Grineva, *Carbohydrate Res.*, 1974, **35**, 235.



(93) R = *p*-substituted (F, Cl, I, OMe, Me) phenyl

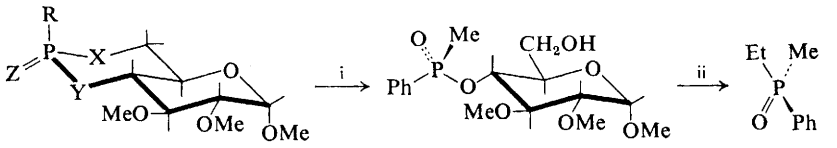
D-glucose 6-phosphate with transition-metal ions (*e.g.* Co^{2+} and Mn^{2+}) have been studied potentiometrically, and the percentages of the different forms present in solution have been determined at various pH values.[266]



Scheme 36

Inch's group has examined the uses of cyclic phosphorus acid esters of carbohydrates in syntheses of optically active phosphorus compounds in investigations of the stereochemistry of displacement reactions at phosphorus; *e.g.* treatment of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside with phosphonic or phosphoric dihalides yielded epimeric pairs of 1,3,2-dioxaphosphorinan-2-ones [*e.g.* (94)], which were separated to give the optically pure forms; the conversion of the methylphosphonate (94) into (−) (*S*)-ethylmethylphenylphosphine oxide (95) is illustrated in Scheme 37.[267] Structural analogues of (94) (X, Y, and Z = O and/or S; R = Me, Ph, OEt, Cl, F, and SR, *etc.*) were also synthesized and the configurations at the phosphorus atom were assigned from i.r. and n.m.r. evidence.[268, 269] In the displacement reactions of these compounds, good leaving groups and weak nucleophiles led to inversion of configuration at the phosphorus

[266] M. Asso and D. Benlian, *Compt. rend.*, 1974, **278**, *C*, 1373.
[267] D. B. Cooper, T. D. Inch, and G. J. Lewis, *J.C.S. Perkin I*, 1974, 1043.
[268] D. B. Cooper, J. M. Harrison, T. D. Inch, and G. J. Lewis, *J.C.S. Perkin I*, 1974, 1049.
[269] J. M. Harrison, T. D. Inch, and G. J. Lewis, *J.C.S. Perkin I*, 1974, 1053.



(94) R = Me; X = Y = Z = O

(95)

Reagents: i, PhMgBr; ii, EtMgBr

Scheme 37

atom, whereas poor leaving groups and strong nucleophiles gave products of retained configuration.[269] The direction of ring-opening was shown to depend on the substitution pattern (*i.e.* the nature of groups X, Y, and Z) and on the stereochemistry at the phosphorus atom.[270]

The syntheses of alkoxyphosphonium salts from the primary hydroxy-groups of such glycosides as methyl α-D-gluco- and -manno-pyranosides, sucrose, and αα-trehalose, and their use in displacement reactions, are shown in Scheme 38; simple secondary alcohols can also react.[271, 271a]

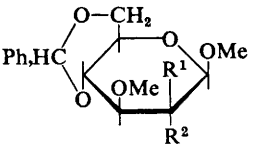
Reagents: i, CCl_4 or $(Me_2CH)_2NCl$; ii, KPF_6; iii, X^-–DMF

Scheme 38

Sulphonates

Mesitylenesulphonyl chloride (trimsyl chloride) has been used for the selective sulphonylation of vicinal diols.[272] Vicinal, *trans*-bis(trimsyloxy)-systems did not yield oxirans on treatment with sodium methoxide (*cf.* the related bis-*O*-toluene-*p*-sulphonates). 1,3,6-Tri-*O*-toluene-*p*-sulphonyl-D-mannitol was obtained on selective sulphonylation of the 3-ester,[273] and *N*-toluene-*p*-sulphonylimidazole was used to sulphonate selectively the hydroxy-group at C-2 of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside.[178]

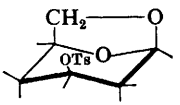
The epimeric mesylates (96) and (97) readily underwent S_N2 displacements with potassium benzoate in DMF, although the corresponding α-anomers were unreactive; the unreactivity of the α-anomers was rationalized in terms of



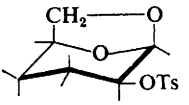
(96) R^1 = H; R^2 = OMs
(97) R^1 = OMs; R^2 = H

[270] D. B. Cooper, J. M. Harrison, T. D. Inch, and G. J. Lewis, *J.C.S. Perkin I*, 1974, 1058.
[271] B. Castro, Y. Chapleur, and B. Gross, *Carbohydrate Res.*, 1974, **36**, 412.
[271a] B. Castro, Y. Chapleur, and B. Gross, *Tetrahedron Letters*, 1974, 2313.
[272] S. E. Creasey and R. D. Guthrie, *J.C.S. Perkin I*, 1974, 1373.
[273] A. B. Zanlungo and G. E. McCasland, *Carbohydrate Res.*, 1974, **38**, 352.

torsional strain and electrostatic and non-bonding interactions in the corresponding transition states.[274] Thus, the widely held belief that the apparent 'unreactivity' of sulphonyloxy-groups at C-2 towards direct displacement can be attributed to the electron-withdrawing effect of the anomeric substituent appears to have no justification. The axial 3-sulphonate (98) has been shown to be more reactive towards substitution and elimination than the isomeric 2-sulphonate (99).[275]



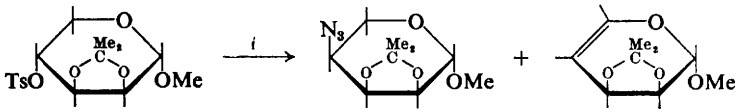
(98)



(99)

A report of considerable interest from a practical standpoint provides an examination of the effects of the solvent, temperature, and the nature of the leaving group on the azide-displacement reactions of a series of carbohydrate sulphonates.[276] It was confirmed that HMPT is the best solvent (HMPT > DMSO > DMF) for the reactions, but such factors as the ease of removal of the solvent may affect the choice. In particular, the presence of up to 10% of water in DMF increased the solubility of azide ions to a sufficient extent to offset the intrinsic rate-depressing effect of water; the other solvents gave the best results when dry. The order of reactivity with respect to the sulphonate group is: *p*-bromobenzenesulphonyl > benzenesulphonyl > toluene-*p*-sulphonyl > methylsulphonyl. The bromo-group of *p*-bromobenzenesulphonates may be displaced by azide ion, but this is not a drawback since the resulting azidobenzenesulphonate still undergoes displacement.

Both substitution and elimination occurred when methyl 2,3-*O*-isopropylidene-4-*O*-toluene-*p*-sulphonyl-α-D-lyxopyranoside reacted with azide ions (see Scheme 39), whereas ring-contraction accompanied the deamination of the



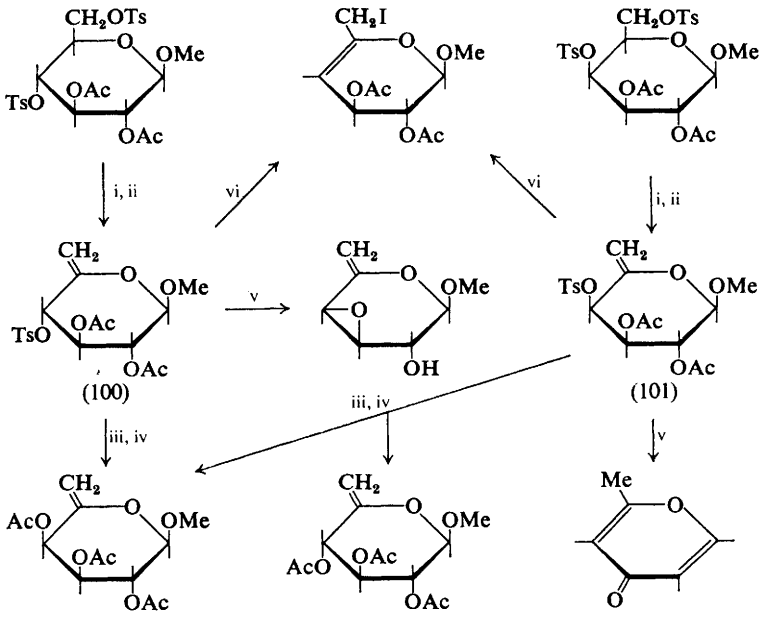
Reagent: i, NaN_3–DMF

Scheme 39

corresponding 4-amino-sugar[277] (see Chapter 8). A number of 4-substituted analogues (the 4-amino-, 4-azido-, 4-bromo-, 4-chloro-, 4-fluoro-, 4-iodo-, and 4-thio-derivatives) of methyl β-D-galactopyranoside have been prepared by way of nucleophilic displacements on either methyl 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside 4-(*p*-bromobenzene)- or 4-trifluoromethane-sulphonates.[278] A series

[274] M. Miljković, M. Gligorijević, and D. Glišin, *J. Org. Chem.*, 1974, **39**, 3223.
[275] J. Pecka, J. Staněk, and M. Černý, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1192.
[276] M.-C. Wu, L. Anderson, C. W. Slife, and J. L. Jensen, *J. Org. Chem.*, 1974, **39**, 3014.
[277] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *Carbohydrate Res.*, 1974, **32**, C7.
[278] A. Maradufu and A. S. Perlin, *Carbohydrate Res.*, 1974, **32**, 261.

of mono-, di-, and tri-sulphonates of methyl β-maltoside have been prepared and used to obtain azido- and amino-deoxy-derivatives.[279] The synthesis and reactions (including S_N2' displacements) of the allylic sulphonates (100) and (101) are shown in Scheme 40.[280]



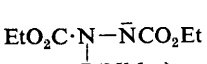
Reagents: i, NaI–Ac_2O; ii, AgF–py; iii, H_2O–py; iv, Ac_2O; v, NaOMe; vi, NaI–Me_2CO

Scheme 40

The use of D-glucopyranosyl toluene-*p*-sulphonates in the Koenigs–Knorr reaction (Chapter 3) and the formation of anhydro-sugars (Chapter 4) have been mentioned already, while the photolytic removal of sulphonyloxy-groups is noted in Chapter 13.

Other Esters

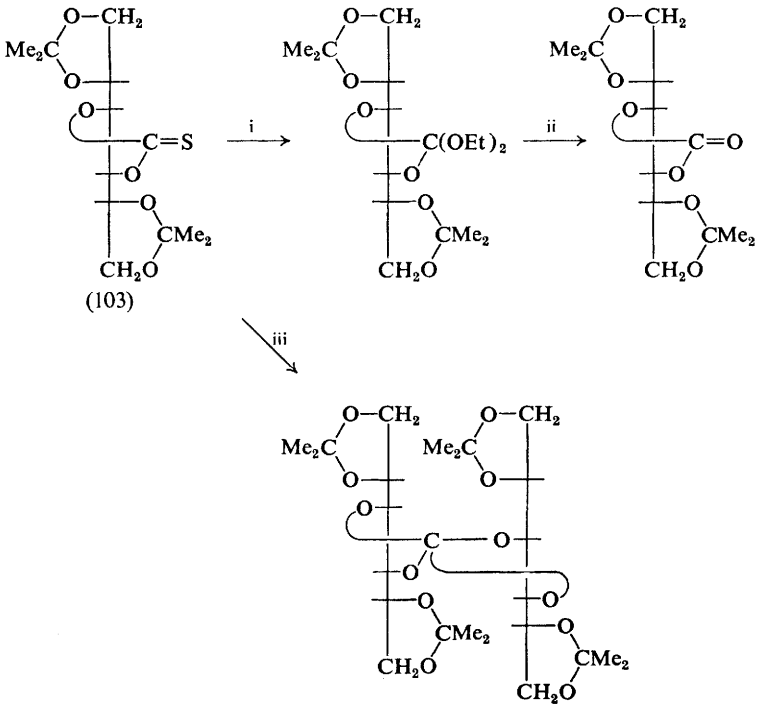
Mixed alkyl-carbohydrate carbonates have been prepared by the reaction of monosaccharides with such betaines as (102), prepared from tris(dimethylamino)-phosphines and dialkyl azodicarboxylates.[281] Treatment of the thionocarbonate



(102)

[279] P. L. Durette, L. Hough, and A. C. Richardson, *J.C.S. Perkin I*, 1974, 97.
[280] M. Brockhaus and J. Lehmann, *Annalen*, 1974, 1675.
[281] G. Grynkiewicz, J. Jurczak, and A. Zamojski, *J.C.S. Chem. Comm.*, 1974, 413.

(103) with either an alcohol (*e.g.* ethanol) or a diol in pyridine containing copper(II) acetate furnished the orthocarbonate (see Scheme 41).[282] L-Arabinose 5-carbamate and D-glucose 6-carbamate have been prepared by way of the appropriate trityl ethers.[255] Phenylcarbamoylation of glycosides has been shown to render the

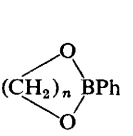


Reagents: *i*, $Cu(OAc)_2$–EtOH; ii, H^+; iii, $Cu(OAc)_2$–1,2:5,6-di-*O*-isopropylidene-D-mannitol

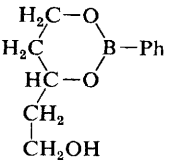
Scheme 41

glycosidic linkage more resistant to hydrolysis, so that only t-butyl hexopyranoside 2,3,4,6-tetraphenylcarbamates were cleaved with acid.[283] A branched-chain sugar carbonate (aldgarose) is mentioned in Chapter 15.

The mass spectra of the products of reaction of benzeneboronic anhydride with a series of α, ω-diols suggested that all are cyclic benzeneboronates (104);



(104) n = 3, 4, 5, 6

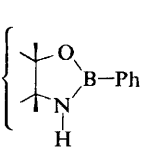


(105)

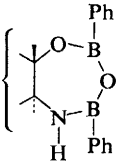
[282] E. I. Stout, B. S. Shasha, D. Trimnell, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1974, **36**, 311.

[283] L. Kenne, B. Lindberg, A. Pilotti, and S. Svensson, *Acta Chem. Scand.* (*B*), 1974, **28**, 559.

pentane-1,3,5-triol formed only a six-membered cyclic boronate (105).[284] The benzene- and butane-boronates of L-arabinose and D-xylose have been prepared in high yield and shown by m.s. and ^{1}H n.m.r. evidence to be the 1,2:3,4- and 1,2:3,5-diboronic esters, respectively, whose conformations resemble those of the related acetals.[285] β-D-Fructopyranose 2,3:4,5-bis- and 1,2-*O*-isopropylidene-α-D-xylofuranose 3,5-monobutane- and benzene-boronates were among other boronic esters reported,[286] and trimethylsilylated derivatives of a number of carbohydrate boronic esters have been studied by g.l.c.–m.s.[287] M.s. was also used to identify the products obtained from the reaction of benzeneboronic anhydride with a series of methyl 2(3)-amino-4,6-*O*-benzylidene-2(3)-deoxy-α-D-aldohexopyranosides; whereas a vicinal *cis*-amino-alcohol afforded a 2-phenyl-1,3,2-oxazaborolidine ring (106), the corresponding *trans*-grouping yielded a 2,4-diphenyl-1,3,5-dioxaza-2,4-diborepine ring (107), even for *trans*-diaxial orientations, when the pyranose ring is required to assume a $B_{2,5}$ conformation.[288]



(106)



(107)

Carbohydrate sulphates have been converted into amidosulphates by sequential reactions with ethoxyacetylene and an appropriate amine.[289, 289a] The hydrazinolysis of carbohydrate sulphates has been reviewed.[290] Desulphation of model peracetylated D-glucose sulphates with thionyl chloride occurred without concomitant migration of the acetyl groups; desulphation has also been achieved with pyridine in DMSO at 96 °C.[291] Solvolytic desulphation of the pyridinium salts of 2-deoxy-2-sulphoamino-D-glucose and D-glucose 6-sulphate has been examined in order to establish optimal conditions for selective *N*-desulphation of heparin; the best results were obtained using DMSO containing 5% of water or methanol.[292] 6-*O*-Methyl-D-galactose 4-sulphate has been referred to earlier in this Chapter.

Nucleoside 5′-nitrates have been prepared in improved yields either directly from nucleosides using 90% nitric acid at −70 °C,[293] or from the 2′,3′-*O*-isopropylidene derivatives using a mixture of acetic anhydride and 60% nitric acid at −30 °C.[294]

[284] E. J. Bourne, I. R. McKinley, and H. Weigel, *Carbohydrate Res.*, 1974, **35**, 141.
[285] P. J. Wood and I. R. Siddiqui, *Carbohydrate Res.*, 1974, **33**, 97.
[286] P. J. Wood and I. R. Siddiqui, *Carbohydrate Res.*, 1974, **36**, 247.
[287] V. N. Rheinhold, F. Wirtz-Peitz, and K. Biemann, *Carbohydrate Res.*, 1974, **37**, 203.
[288] I. R. McKinley, H. Weigel, C. B. Barlow, and R. D. Guthrie, *Carbohydrate Res.*, 1974, **32**, 187.
[289] N. K. Kochetkov, A. I. Usov, and V. V. Deryabin, *Doklady Akad. Nauk S.S.S.R.*, 1974, **216**, 97.
[289a] N. K. Kochetkov, A. I. Usov, and V. V. Deryabin, *Zhur. obshchei Khim.*, 1974, **44**, 904.
[290] T. Suami, *Yuki Gosei Kagaku Kyokai Shi*, 1973, **31**, 791.
[291] A. F. Pavlenko, N. I. Belogortseva, A. I. Kalinovskii, and Yu. S. Ovodov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1593.
[292] K. Nagasawa and Y. Inoue, *Carbohydrate Res.*, 1974, **36**, 265.
[293] F. W. Lichtenthaler and H. J. Müller, *Angew. Chem. Internat. Edn.*, 1973, **12**, 752.
[294] F. W. Lichtenthaler and H. J. Müller, *Synthesis*, 1974, 199.

7
Halogenated Sugars

Glycosyl Halides

A number of reactions involving glycosyl halides are noted in Chapter 3, where attention is drawn to a report describing the use of glycosyl iodides.

β-Glycopyranosyl chlorides have been prepared by treating the corresponding α-chlorides with silver perchlorate in ether, followed by tetraethylammonium chloride in acetonitrile.[295] In a related paper it was shown that treatment of β-D-glucopyranose penta-acetate with titanium tetrachloride gave initially a complex of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride and titanium acetoxytrichloride ($TiCl_3OAc$); the titanium salt of the complex then catalysed the anomerization to the α-chloride.[296] Similar results were obtained in the D-galactose series. A report has appeared describing the preparation of crystalline 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl chloride from the tetra-acetate using anhydrous hydrogen chloride in dichloromethane at 0 °C.[297] Treatment of L-fucose with sulphuryl chloride at low temperature furnished mainly 2,3,4-tri-*O*-chlorosulphonyl-β-L-fucopyranosyl chloride and a small proportion of the α-anomer; both chlorides adopted the $^1C_4$ chair conformation in solution.[298] Methanolysis of the β-chloride gave methyl 2,3,4-tri-*O*-chlorosulphonyl-α-L-fucopyranoside, with the formation of a small proportion only of the β-glycoside (see Chapter 3 for a related report).

Treatment of peracetylated hexoses with aluminium bromide in chloroform yielded both glycosyl chlorides and bromides as a result of halogen exchange between the salt and the solvent.[299] Several crystalline glycosyl halides bearing an azido-group at C-4 have been synthesized and used in the preparation of cardiac glycosides, antibiotics, and nucleosides containing 4-amino-4-deoxy-sugar residues.[300]

Phillips and his colleagues have extended their kinetic studies of the displacement reactions of glycosyl halides by examining the bromide-exchange reactions and hydrolyses of a series of benzoylated glycosyl bromides.[301] The effects of exchanging acetyl groups by benzoyl groups were examined.

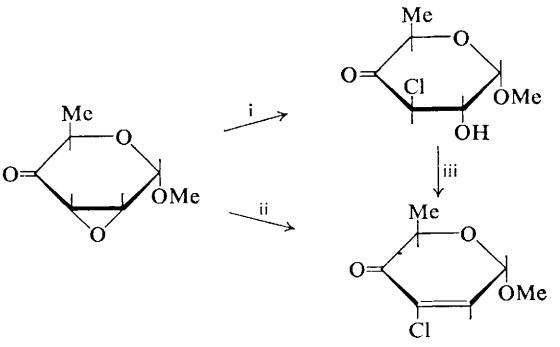
[295] K. Igarashi, T. Honma, S. Mori, and J. Irisawa, *Carbohydrate Res.*, 1974, **38**, 312.
[296] Z. Csuros, G. Deak, L. Fenichel, S. Holly, and J. Palinkas, *Acta Chim. Acad. Sci. Hung.*, 1974, **80**, 193 (*Chem. Abs.*, 1974, **80**, 146 429v).
[297] R. A. Earl and L. B. Townsend, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1974, **1**, 177.
[298] J.-R. Pougny, P. Sinaÿ, and G. Hajdukovic, *Carbohydrate Res.*, 1974, **34**, 351.
[299] T. Lin and R. E. Harmon, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1974, **1**, 109.
[300] C. L. Stevens, G. H. Ransford, J. Němec, J. M. Cahoon, and P. M. Pilkai, *J. Org. Chem.*, 1974, **39**, 298.
[301] M. J. Duffy, G. Pass, and G. O. Phillips, *J.C.S. Perkin II*, 1974, 1466.

Other Halogenated Derivatives

Interest in the biochemistry of fluoro-sugars has continued. The 2-, 3-, 4-, and 6-deoxyfluoro-derivatives of D-galactose have been examined as substrates for yeast galactokinase, and the nature of the associative interactions between the sugars and the enzyme was deduced.[302] The transport of 3-deoxy-3-fluoro-D-glucose across human-erythrocyte membranes has been compared with that of several related sugars.[303] Several 3′-deoxy-3′-fluoro-nucleoside derivatives are noted in Chapter 21.

Only the primary hydroxy-group of methyl pentofuranosides underwent displacement on treatment with sulphuryl chloride, thereby providing a convenient route to 5-chloro-5-deoxypentoses.[304] Sulphuryl chloride reacted with methyl β-D-glucopyranoside to give, after dechlorosulphation, methyl 4,6-dichloro-4,6-dideoxy-β-D-galactopyranoside (22%) and methyl 3,6-dichloro-3,6-dideoxy-β-D-allopyranoside (50%).[305] The methanesulphonyl chloride–DMF reagent has been shown to cause extensive, but selective, chlorination at secondary hydroxy-groups of glycopyranosides; *e.g.* methyl β-D-glucopyranoside afforded a 2 : 1 mixture of methyl 3,6-dichloro-3,6-dideoxy-β-D-allopyranoside and methyl 4,6-dichloro-4,6-dideoxy-β-D-galactopyranoside (see also ref. 305), whereas the α-glycoside gave mainly the 4,6-dichloro-galactopyranoside.[306] Similar treatment of methyl β-maltoside gave 6,6′-di-, 3,6,6′-tri-, and 3,4′,6,6′-tetra-chloro-derivatives.

Paulsen and his colleagues have investigated the chemistry of anhydro-sugar derivatives of a number of pyranosiduloses, and have obtained a number of novel chloro-compounds (Scheme 42); bromo- and iodo- analogues of the enone

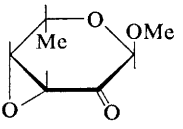


(108)

Reagents: i, LiCl–Me_2CO; ii, LiCl–THF; iii, Ac_2O

Scheme 42

[302] P. Thomas, E. M. Bessell, and J. H. Westwood, *Biochem. J.*, 1974, **139**, 661.
[303] G. J. Riley and N. F. Taylor, *Biochem. J.*, 1973, **135**, 773.
[304] B. Achmatowicz, W. A. Szarek, J. K. N. Jones, and E. H. Williams, *Carbohydrate Res.*, 1974, **36**, C14.
[305] D. M. Dean, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1974, **33**, 383.
[306] R. G. Edwards, L. Hough, A. C. Richardson, and E. Tarelli, *Carbohydrate Res.*, 1974, **35**, 111.



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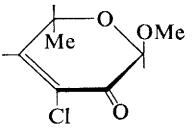


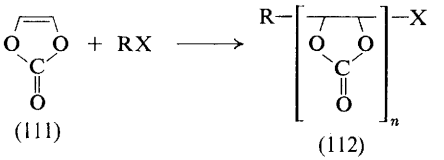
(110)

(108) were also obtained. Treatment of the 2-uloside derivative (109) with lithium chloride in THF gave the chloro-enone (110).[307]

^{13}C N.m.r. studies of chlorodeoxy-sugars have proved extremely helpful in elucidating the structures of these compounds.[308]

A novel method of bromination has been reported that could find use in carbohydrate chemistry; under mild conditions, triphenylphosphine dibromide (Ph_3PBr_2) in DMF converted primary alcohols into the corresponding bromides and secondary alcohols into the formate esters.[309] Jacobsen and Pedersen have prepared a number of bromo-sugars in the course of their work on benzoxonium ions (see Scheme 25),[203] and syntheses of 3-bromo-3-deoxy- and 3-chloro-3-deoxy-maltosides [185] have been referred to already in Chapter 4.

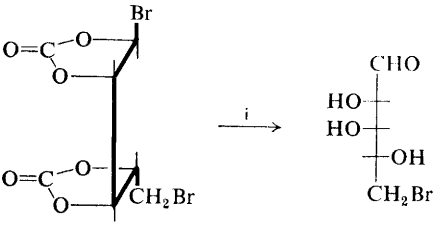
Halogenated sugars have been encountered in a novel approach to the total synthesis of monosaccharides. Thus, treatment of vinylene carbonate (111)



(111) (112)

RX = CCl_4, CBr_4, $CHCl_3$, CH_2Br_2, *etc.*

with polyhalogenomethanes in the presence of radical initiators yielded telomers of the general structure (112), several of which (n = 2, 3, or 4) were isolated by chromatography and characterized.[310] These compounds offered a means of synthesizing racemic pentoses (Scheme 43), heptoses, and nonoses, respectively, by the most direct route available from non-carbohydrate precursors.



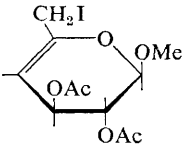
Reagent: 0.5M-H_2SO_4

Scheme 43

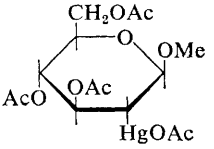
[307] H. Paulsen, K. Eberstein, and W. Koebernick, *Tetrahedron Letters*, 1974, 4377.
[308] W. A. Szarek, D. M. Vyas, S. D. Gero, and G. Lukacs, *Canad. J. Chem.*, 1974, **52**, 3394.
[309] R. K. Boeckman, jun. and B. Ganem, *Tetrahedron Letters*, 1974, 913.
[310] T. Tamura, T. Kunieda, and T. Takizawa, *J. Org. Chem.*, 1974, **39**, 38.

An investigation of the reactions of 6-deoxy-hex-5-enopyranosides has led to a synthesis of the 6-iodo-compound (113) (see Scheme 40 for details).[280]

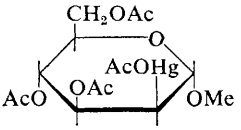
The acetoxymercuri-group in each of the isomeric glycosides (114) and (115) was displaced by iodine with both retention and inversion of configuration at



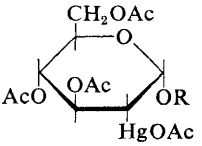
(113)



(114)



(115)



(116) R = H
(117) R = Ac

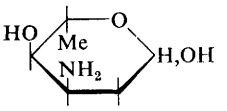
C-2.[311] Similar replacement was observed with 2-acetoxymercuri-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (116), although in the iodinolysis of 2-acetoxymercuri-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (117) in methanol, replacement at C-2 was accompanied by solvolysis of the 1-acetoxy-group.

[311] S. Honda and K. Takiura, *Carbohydrate Res.*, 1974, **34**, 45.

8
Amino-sugars*

Natural Products

Ristosamine, a component of the antibiotic ristamycin, has been identified as 3-amino-2,3,6-trideoxy-L-*ribo*-hexose (118).[312, 312a]



(118)

Synthesis

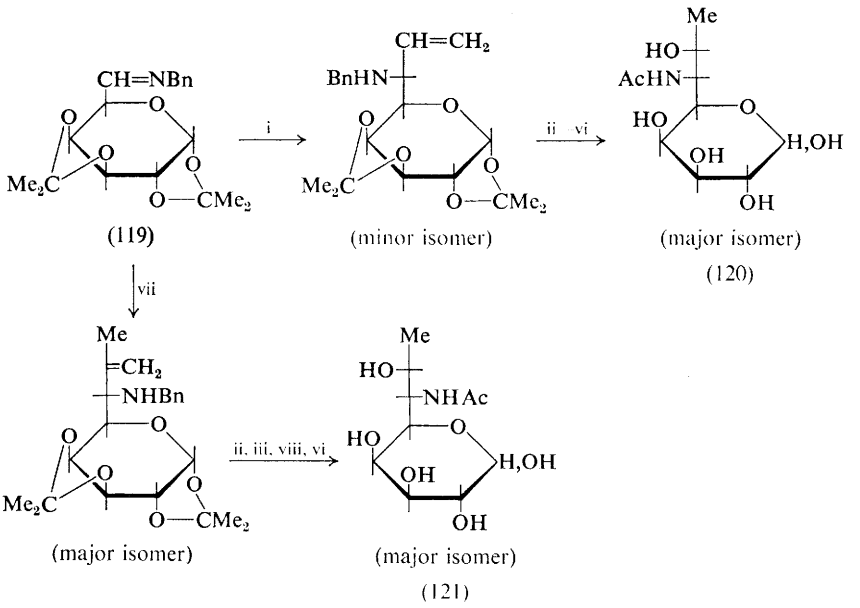
The reaction of the aldimine derivative (119) with Grignard reagents has been used in syntheses of *N*-acetyl-lincosamine (120)[313] and *N*-acetyl-6-epilincosamine (121),[314] as shown in Scheme 44; *N*-acetyl-7-epilincosamine was similarly obtained from the minor product of the Grignard reaction. Syntheses of celestosamine derivatives and modified lincosamines are referred to in Chapter 20.

Following earlier syntheses of D-desosamine and its racemic form, an approach to the L-isomer (122) from the nitro-olefin (123) has now been devised.[315] The nitromethane–dialdehyde procedure has been used in the synthesis of methyl 3-amino-3,6-dideoxy-β-D-hexopyranosides; the principal nitro-sugar formed initially has the *gluco*-configuration, but isomerization with alkali furnished a mixture of nitro-sugars in which the *galacto*-isomer predominated.[316]

Methyl 2-acetamido-2,6-dideoxy-α-L-galactopyranoside was obtained as the major product following reduction, with lithium aluminium hydride, of the oxime (124) prepared by the nitrosyl chloride–glycal procedure.[317] 3-Acetamido-3-deoxy-D-glucose was obtained *via* similar reduction of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose oxime,[318] and details of the syntheses of methyl

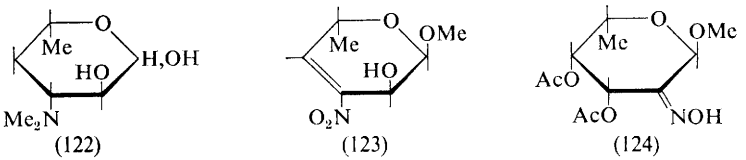
[312] I. A. Spiridonova, N. N. Lomakina, F. Sztaricskai, and R. Bognar, *Antibiotiki*, 1974, **19**, 400 (*Chem. Abs.*, 1974, **81**, 91 882a).
[312a] R. Bognar, F. Sztaricskai, M. E. Munk, and J. Tamas, *J. Org. Chem.*, 1974, **39**, 2971.
[313] T. Atsumi, T. Fukumaru, T. Ogawa, and M. Matsui, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2621.
[314] T. Atsumi, T. Fukumaru, and M. Matsui, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2627.
[315] H. H. Baer and C.-W. Chiu, *Canad. J. Chem.*, 1974, **52**, 122.
[316] K. Čapek, J. Staněk, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1462.
[317] P. J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand.*, 1974, **B28**, 1104.
[318] I. Jezo, *Chem. Zvesti*, 1973, **27**, 681 (*Chem. Abs.*, 1974, **80**, 96 265r).

* See also Part I, Chapter 20.



Reagents: i, CH_2=CHMgCl; ii, Ac_2O; iii, O_3; iv, MeMgBr; v, Na–NH_3; vi, H_3O^+; vii, CH_2=CMeMgBr; viii, $NaBH_4$

Scheme 44



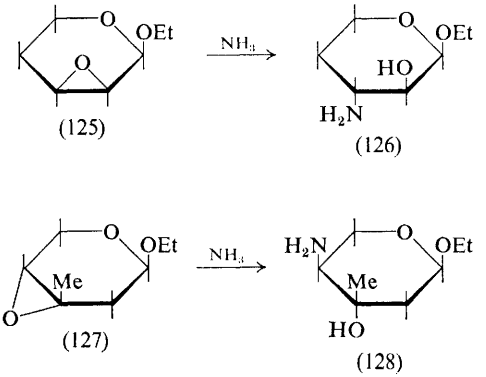
4-amino-4,6-dideoxy-α- and -β-D-allopyranosides from osulose oximes have appeared[319] (see Vol. 6, p. 61).

Displacement of sulphonates or halogens with azide ions has remained a popular approach to the synthesis of amino-sugars. Details of a synthesis of 4-amino-4-deoxy-D-galactose from methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methylsulphonyl-α-D-glucopyranoside (see Vol. 2, p. 81) indicated that this is the best route to this amino-sugar.[320] Halogenated sugars have furnished substrates for syntheses of 6-amino-2,6-dideoxy-2-fluoro-D-glucose[321] and methyl 3-acetamido-3,6-dideoxy-β-D-gluco- and -manno-pyranosides,[322] providing an alternative to the nitromethane route for the latter compounds. 1,6-Anhydro-β-cellobiose has also been converted into 6′-acetamido-6′-deoxycellobiose and 4′-acetamido-4′-deoxylactose by sequences involving azide displacements on sulphonate

[319] C. L. Stevens, K. K. Balasubramanian, C. P. Bryant, J. B. Filippi, and P. M. Pillai, *J. Org. Chem.*, 1973, **38**, 4311.
[320] F. W. Lichtenthaler and P. Heidel, *J. Org. Chem.*, 1974, **39**, 1457.
[321] J. Pacák, J. Hriňák, and M. Černý, *Coll. Czech. Chem. Comm.*, 1974, **39**, 3332.
[322] J. Staněk, K. Čapek, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1479.

esters.[323] Similar displacements have also furnished 6′-mono- and 6,6′- and 3,6′-di-amino-disaccharides from sulphonate esters of methyl β-maltoside.[279]

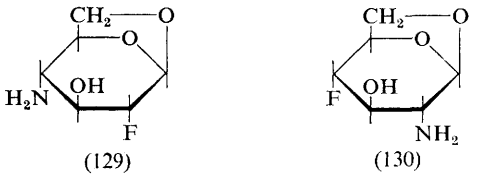
Treatment of oxirans with either ammonia or azide ions has been adopted by several groups as a method for the synthesis of amino-sugars. Standard transformations on ethoxy- and 4-methylethoxy-pyrans have yielded derivatives of 3-amino-3,4-dideoxy-DL-*threo*-pentose (126) and 4-amino-2,4-dideoxy-3-*C*-methyl-DL-*threo*-pentose (128) from the corresponding oxirans (125) and (127) (Scheme 45).[324] The fluorinated amino-sugar derivatives (129) and (130) were



(125) (126) (127) (128)

All compounds are racemates

Scheme 45



(129) (130)

similarly obtained by ring-opening of appropriate oxirans with ammonia.[325] A reinvestigation of the reaction of ammonia with methyl 2,3-anhydro-α-D-ribofuranoside and its 5-deoxy-derivative afforded the products shown in Scheme 46.[326] The oxiran (131) reacted with sodium azide to give, after catalytic hydrogenation, a derivative (132) of 3-amino-2,3,6-trideoxy-L-*arabino*-hexose, an analogue of daunosamine.[326a] 2-Amino-1,6-anhydro-2-deoxy-β-D-mannopyranose (135) has been synthesized from the dianhydro-sugar (133) by way of the aziridine (134), as shown in Scheme 47.[327]

[323] Y. Okamuri, M. Haga, and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2538.

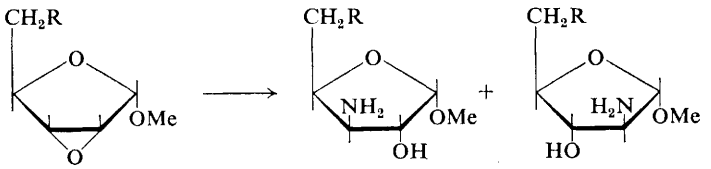
[324] V. B. Mochalin, Z. I. Smolina, A. N. Kornilov, and B. V. Unkovskii, *Khim. Khim. Tekhnol., Trudy Yubileinoi. Konf., Posvyashch.* 70-*Letiyu Inst.* (*Mosk. Inst. Tonkoi Khim. Tekhnol.*) 1970, Moscow, 1972, p. 154.

[325] J. Pacák, P. Drašar, D. Štropová, M. Černý, and M. Buděšínský, *Coll. Czech. Chem. Comm.*, 1973, **38**, 3936.

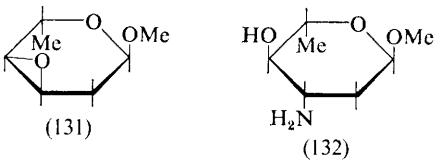
[326] J. A. Montgomery, M. C. Thorpe, S. D. Clayton, and H. J. Thomas, *Carbohydrate Res.*, 1974, **32**, 404.

[326a] S. K. Gupta, *Carbohydrate Res.*, 1974, **37**, 381.

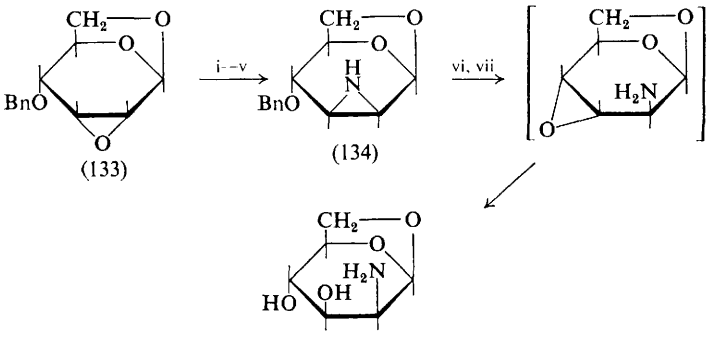
[327] M. Černý, T. Elbert, and J. Pacák, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1752.



Scheme 46



The reaction of *O*-benzyltryptamine with D-glucuronic acid occurred with Amadori rearrangement to give a derivative of 1-amino-1-deoxy-D-fructuronic acid, which yielded the cyclic amide (136) on acetylation.[328] Derivatives (137) of 2-amino-2-deoxy-D-(or L)-gluconic acid have been prepared by treating 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-(or L)-arabinose with cyclohexyl isocyanide and

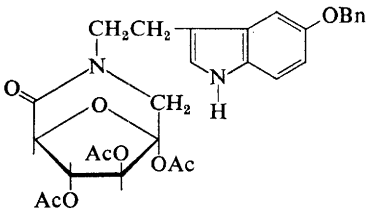


Reagents: i, NaN_3; ii, MsCl–py; iii, Zn–HCl; iv, *p*-$NO_2C_6H_4COCl$; v, $NaOPr^i$; vi, H_2–Pd; vii, KOH

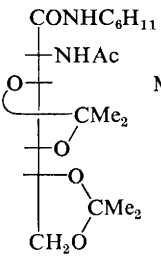
Scheme 47

ammonium acetate in the Ugi reaction.[329] A number of ether and ester derivatives of methyl 2-acetamido-2-deoxy-β-D-glucofuranoside have been synthesized for comparison with the corresponding pyranosides [330] and as starting materials for the preparation of the muramic acid dithioacetal (138), a potential intermediate

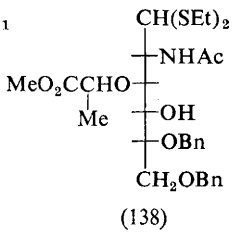
[328] M. M. Vigdorchik, R. P. Olad'ko, N. P. Kostyuchenko, and N. N. Suvorov, *Zhur. org. Khim.*, 1974, **10**, 1256.
[329] A. I. Polyakov, N. N. Aseeva, and V. G. Vezrukova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1597.
[330] J.-C. Jacquinet and P. Sinaÿ, *Carbohydrate Res.*, 1974, **32**, 101.



(136)

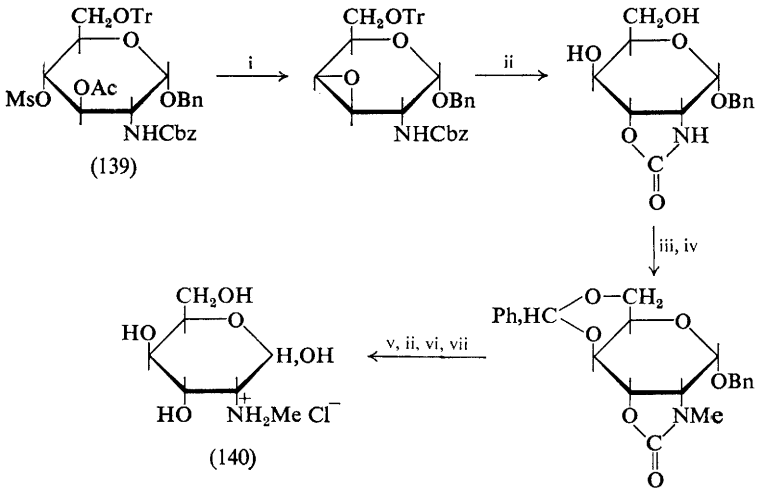


(137)



(138)

for the synthesis of disaccharides.[330a] 2-Deoxy-2-methylamino-D-gulose (140) has been synthesized from the benzyl 2-amino-2-deoxy-glucopyranoside derivative (139), as shown in Scheme 48.[331]



(139)

(140)

Reagents: i, $NaOPr^i$; ii, AcOH; iii, PhCHO–$ZnCl_2$; iv, MeI–BaO; v, KOH–EtOH; vi, Ac_2O–py; vii, H_3O^+

Scheme 48

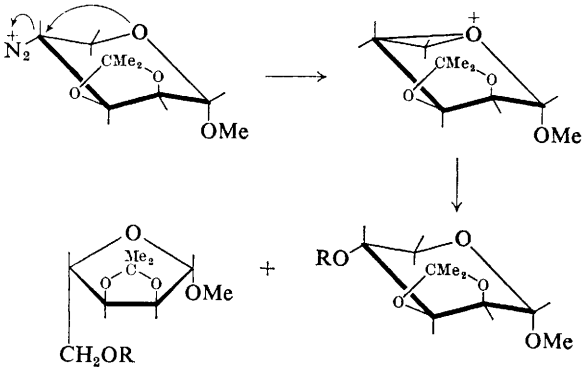
Analogues of 2′-deoxythymidine 5′- and 3′-phosphates having amino-groups replacing the hydroxy-groups at C-3′ and C-5′, respectively, have been prepared by way of displacements with azide ions;[332] a number of *N*-substituted derivatives so prepared inhibited mammalian-cell *exo*-ribonuclease.

Other nucleosides containing amino-groups are mentioned in Chapter 21, and the synthesis of branched-chain amino-sugars is dealt with in Chapter 15. Syntheses of the following amino-sugars are referred to in other chapters: methyl α-DL-mycaminoside (Chapter 13), 3,6-anhydro-derivatives of 2-amino-2-deoxy-D-glucose (Chapter 4), and derivatives of 5-amino-5-deoxy-uronic and -saccharic acids (Chapter 17).

[330a] J.-C. Jacquinet and P. Sinaÿ, *Carbohydrate Res.*, 1974, **34**, 139.
[331] H. M. Noorzad and P. H. Gross, *Carbohydrate Res.*, 1973, **31**, 229.
[332] R. P. Glinski, M. S. Khan, R. L. Kalamas, and M. B. Sporn, *J. Org. Chem.*, 1973, **38**, 4299.

Reactions

Re-examination of the deamination of methyl 2-amino-2-deoxy-α-D-glucopyranoside has shown that 2,5-anhydro-D-mannose and methyl 2-deoxy-2-*C*-formyl-α-D-*arabino*-pentofuranoside are formed in the ratio of 3 : 1; the products result from migration of the O-5—C-1 and C-3—C-4 bonds, respectively, consistent with a carbonium-ion rearrangement.[333] Details of the deamination of methyl 5-amino-5,6-dideoxy-2,3-*O*-isopropylidene-α-L-talofuranoside and the corresponding β-D-allofuranoside have been published (see Vol. 6, p. 64); differences between the two sets of products were based on the assumption that the carbonium ion reacts in the initial conformation before significant interconversion into other conformers can take place.[334] Methyl 4-amino-4-deoxy-2,3-*O*-isopropylidene-α-D-lyxopyranoside underwent deamination with nitrous acid in aqueous acetic acid to give a 3 : 2 mixture of D-lyxopyranoside and ring-contracted L-ribofuranoside derivatives, which are considered to be derived from an intermediate bicyclic oxonium ion (see Scheme 49) (*cf.* the homologous rhamnoside; Vol. 4, p. 64).[277] The deamination of 6-amino-sugars with ninhydrin, to give dialdose derivatives, is referred to in Chapter 16.



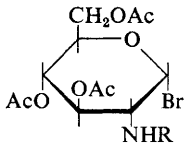
R = H or Ac

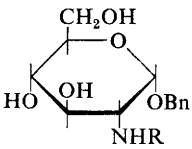
Scheme 49

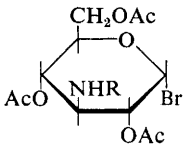
The condensation between the 2-amino-2-deoxy-D-glucose derivatives (141) and (142) yielded primarily the α-(1 → 6)-linked disaccharide; similarly, α- and β-(1 → 6)-linked 3,3′-diamino-disaccharides were obtained by way of the intermediates (143) and (144).[335]

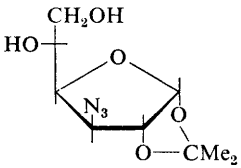
In a study of the hydrolysis of *N*-sulphoamino-compounds having a neighbouring hydroxy- or *O*-sulphate group, the relatively rapid hydrolysis of 2-deoxy-2-sulphoamino-D-glucose was attributed to the combined effects of the hydroxy-groups, the pyranose-ring structure, and the ring-oxygen atom.[336]

[333] C. Erbing, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 3699.
[334] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *Carbohydrate Res.*, 1974, **35**, 55.
[335] W. Meyer zu Reckendorf, B. Radatus, E. Bischof, and R. Weber, *Chem. Ber.*, 1974, **107**, 869.
[336] Y. Inoue and K. Nagasawa, *Carbohydrate Res.*, 1973, **31**, 359.


(141)


(142)

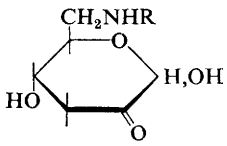

(143)

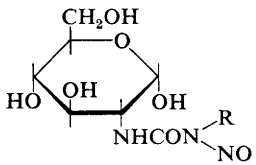

(144)

R = 2,4-$(NO_2)_2$·C_6H_3

Browning reactions involving a number of 6-amino-6-deoxy-D-glucose derivatives and various amines have been investigated.[337] Primary aromatic amines and piperidine gave glycosylamine derivatives, whereas secondary and tertiary amines afforded products arising from dehydration; more-basic primary amines (*e.g.* 6-benzylamino-6-deoxy-D-glucose) also gave products of this type [*e.g.* (145)].

Treatment of 2-amino-2-deoxy-D-glucose with either methyl or ethyl chlorocarbonate and sodium carbonate in methanol has provided a one-step synthesis


R = *p*-C_6H_4Me or Bn
(145)


R = Et, Pr^n, Bu^n, Bn
(146)

of the methyl 2-deoxy-2-methoxy- or -ethoxy-carbonylamino-D-glucopyranosides; the α-glycoside (83%) was obtained when the reaction was carried out in boiling methanol, whereas the β-glycoside (65%) was formed in methanol at ambient temperature.[338] Reaction of 2-amino-2-deoxy-D-glucose with *N*-alkyl-*N*-nitrosocarbamoyl azides has furnished a series of modified streptozotocines (146) (streptozotocin, R = Me).[339]

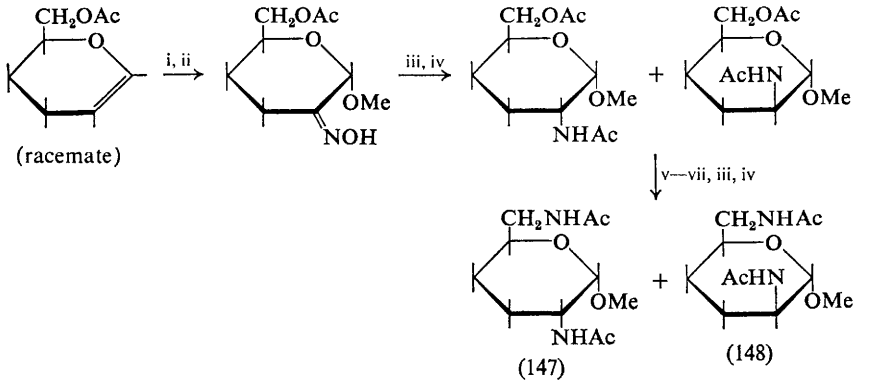
The thermolysis of amino-sugars has been studied; glycosylamines are produced initially and are then degraded by dehydration *etc.*, often with charring.[340]

[337] R. Neumann and G. Henseke, *Z. Chem.*, 1974, **14**, 155.
[338] S. Otani, *Bull. Chem. Soc. Japan*, 1974, **47**, 781.
[339] A. Meier, F. Stoos, D. Martin, G. Buyuk, and E. Hardegger, *Helv. Chim. Acta*, 1974, **57**, 2622.
[340] F. Shafizadeh, G. D. McGinnis, P. A. Susott, and M. H. Meshreki, *Carbohydrate Res.*, 1974, **33**, 191.

The partial esterification of derivatives of amino-sugars is reported in Chapter 6, and ^{15}N n.m.r. spectroscopy of 6-deoxy-6-phthalimidohexose derivatives and 220 MHz ^1H n.m.r. spectroscopy of the products arising from Amadori rearrangement of D-glucose are mentioned in Chapter 23.

Di- and Poly-amino-sugars

Details of syntheses of 2-acetamido-2,6-dideoxy-D-glucose, 2-acetamido-2,6-dideoxy-D-galactose, and 2,4-diacetamido-2,4,6-trideoxy-D-glucose have been published[341] (see Vol. 7, p. 75). Methyl *N*-acetyl-α-DL-purpurosaminide C (147) and the corresponding derivative (148) of DL-epipurpurosamine C have been obtained from 2-acetoxy-3,4-dihydro-2*H*-pyran by the route outlined in Scheme 50.[342]



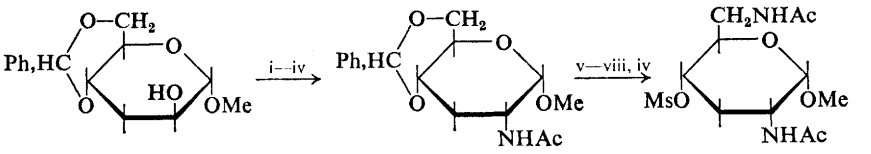
Reagents: i, NOCl; ii, MeOH–py; iii, H_2–Pd; iv, Ac_2O; v, basic resin; vi, TsCl–py; vii, NaN_3

Scheme 50

Derivatives of 3,6-diamino-3,6-dideoxy-D-allose have been prepared from 1,2-*O*-isopropylidene-3,6-di-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose by way of displacements with ammonia or amines.[343] In addition to the products reported earlier (see Vol. 7, p. 75), benzyl 3,4-diazido-3,4,6-trideoxy-β-L-glucopyranoside (as the 3,5-dinitrobenzoate) has been isolated from the reaction of sodium azide in DMF with benzyl 2,3-anhydro-6-deoxy-4-*O*-methylsulphonyl-β-L-gulopyranoside; the transformation of the diazido-derivative into 3,4-diacetamido-3,4,6-trideoxy-L-glucose was also described.[344]

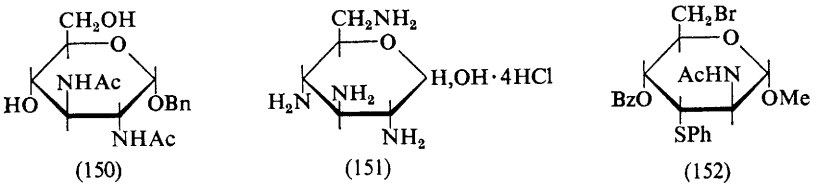
Nebrosamine, a naturally occurring diamino-sugar, has been identified as 2,6-diamino-2,3,6-trideoxy-D-*ribo*-hexose by comparison of a 4-methanesulphonate derived therefrom with the corresponding derivative (149) obtained by the route shown in Scheme 51.[345] Neamine has been converted into 3′,4′-dideoxy- and 3′- and 4′-*O*-methyl-neamines by standard transformations.[346]

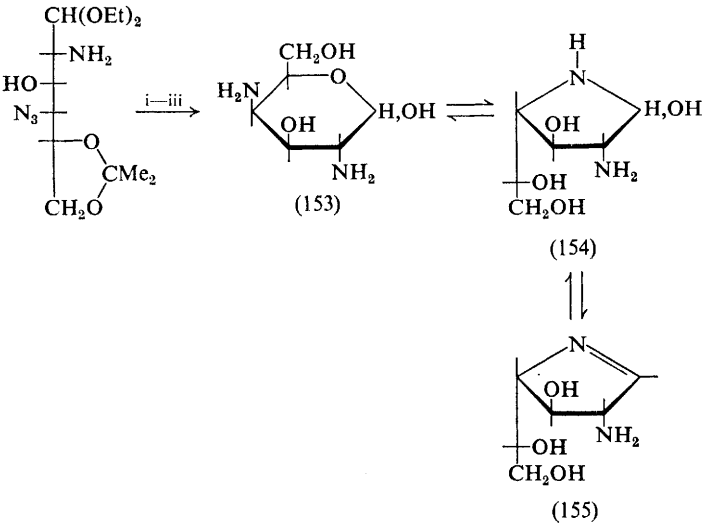
[341] A. Liav, J. Hildesheim, U. Zehavi, and N. Sharon, *Carbohydrate Res.*, 1974, **33**, 217.
[342] J. S. Brimacombe, I. Da'Aboul, and L. C. N. Tucker, *J.C.S. Perkin I*, 1974, 263.
[343] I. Jezo, *Chem. Zvesti*, 1973, **27**, 684 (*Chem. Abs.*, 1974, **80**, 96 266s).
[344] A. Liav and N. Sharon, *Carbohydrate Res.*, 1974, **37**, 248.
[345] C. L. Brewer and R. D. Guthrie, *J.C.S. Perkin I*, 1974, 657.
[346] T. Jikihara, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 3507.



Reagents: i, RuO_4; ii, NH_2OH; iii, $LiAlH_4$; iv, Ac_2O; v, 60% AcOH; vi, MsCl–py; vii, NaN_3 (80 °C); viii, H_2–Pt

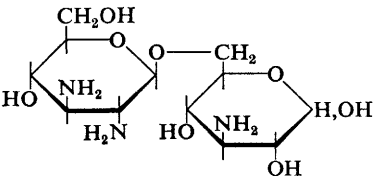
Scheme 51

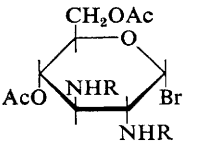




Reagents: i, HCl; ii, H_2–Pd; iii, HO^-

Scheme 52





2,3,4,6-Tetra-amino-2,3,4,6-tetradeoxy-D-glucose [as the hydrochloride (151)] has been synthesized from the 2,3-diamino-sugar derivative (150) by first inverting the configuration at C-4, followed by standard displacements with azide ion *etc.*, on the derived 4,6-disulphonate [347] (*cf.* Vol. 4, p. 66).

A synthesis aimed at the amino-cyclitol antibiotic kasugamine was thwarted by the failure of the compound (152) to undergo desulphurization with Raney nickel.[348] 2,4-Diamino-2,4-dideoxy-D-galactose, prepared as shown in Scheme 52, has been shown to exist in solution as an equilibrium mixture of the cyclic forms (153), (154), and (155), with (154) predominating.[349] Disaccharides [*e.g.* (156)] incorporating a 2,3-diamino-2,3-dideoxy-D-glucopyranosyl residue have been synthesized from the glycosyl bromide (157) by standard procedures.[350] A number of the reactions of 2,3-diamino-2,3-dideoxy-L-ascorbic acid have been investigated.[351]

Syntheses of diamino-sugars from derivatives of nitro-olefins and epimines are referred to in Chapter 10.

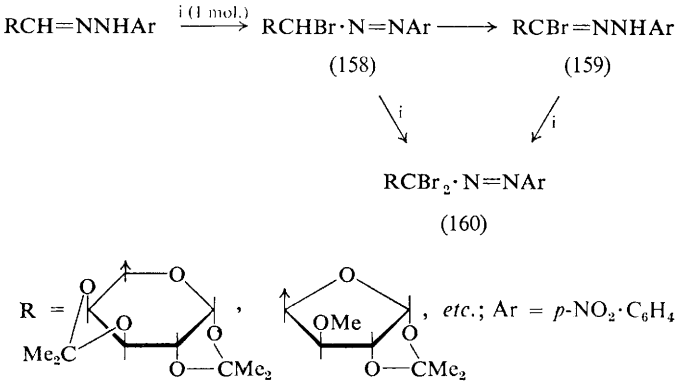
[347] W. Meyer zu Reckendorf and N. Wassiliadou-Micheli, *Chem. Ber.*, 1974, **107**, 1188.
[348] S. Hanessian and R. Masse, *Carbohydrate Res.*, 1974, **35**, 175.
[349] H. Paulsen and U. Grage, *Chem. Ber.*, 1974, **107**, 2016.
[350] W. Meyer zu Reckendorf and R. Weber, *Chem. Ber.*, 1974, **107**, 2585.
[351] B. Gross, M. El Sekily, S. Mancy, and H. S. El Khadem, *Carbohydrate Res.*, 1974, **37**, 384.

9

Hydrazones, Osazones, and Related Compounds

Whereas D-glucose gave the expected osazone with 2,4-dinitrophenylhydrazine, 3-ketoses (*e.g.* D-*altro*-3-heptulose) have yielded the corresponding 1-deoxy-(2,4-dinitrophenylosazones), which are convenient crystalline derivatives for characterization of the parent sugars; formation of the 1-deoxy-derivative was suggested to involve dehydration, to give the 1-deoxy-2,3-diulose, as the initial step.[352, 352a]

Bromination of the *p*-nitrophenylhydrazones of *aldehydo*-sugars afforded *gem*-bromo-azo-derivatives (158), which yielded hydrazonoyl bromides (159) on prototropic rearrangement;[353] both classes of compound furnished dibromo-azo-derivatives (160) on further bromination (Scheme 53).[354]

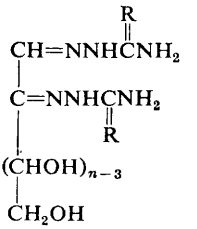


Reagent: i, Br_2–AcOH

Scheme 53

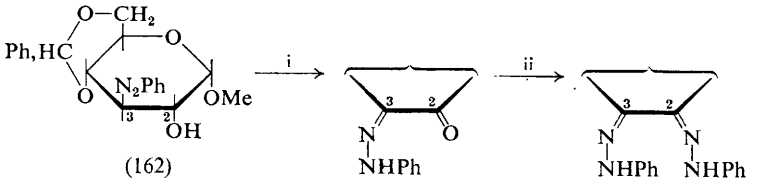
A series of bis(guanylhydrazones) (161; R = NH) and bis(thiosemicarbazones) (161; R = S) of aldosuloses have been prepared; the copper chelate of D-*arabino*-hexosulose bis(thiosemicarbazone) and the copper and palladium chelates of 6-deoxy-L-*arabino*-hexosulose bis(thiosemicarbazone) showed antitumour activity in the KB cell-culture test-system.[355]

[352] T. Okuda, S. Saito, and K. Uobe, *Tetrahedron*, 1974, **30**, 1187.
[352a] T. Okuda, S. Saito, K. Watanabe, and C. Hieda, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2202.
[353] J. M. J. Tronchet, B. Baehler, F. Perret, and J. Poncet, *Carbohydrate Res.*, 1974, **34**, 331.
[354] J. M. J. Tronchet, B. Baehler, J. Poncet, F. Perret, and A. Jotterand, *Carbohydrate Res.*, 1974, **34**, 376.
[355] F. H. H. Carlsson, A. J. Charlson, and E. C. Watton, *Carbohydrate Res.*, 1974, **36**, 359.



(161)

Radicals generated from inosose phenylhydrazones and osazones with alkali in oxygenated solutions of DMSO have been investigated; stable radical-anions, in which the phenylhydrazone moiety remained intact, were produced from inosose phenylhydrazones.[356] The 2,3-diulose 3-phenylhydrazone derivative (163), obtained by oxidation of methyl 4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucopyranoside (162) with the Pfitzner–Moffatt reagent, yielded the phenylosazone (164) on treatment with phenylhydrazine (Scheme 54).[357]



(162) (163) (164)

Reagents: i, DCC–DMSO; ii, $PhNHNH_2$

Scheme 54

^1H N.m.r. spectroscopic studies of the conformations of inosose phenylosotriazoles are mentioned in Chapter 23.

[356] A. J. Fatiadi, in 'Carbohydrates in Solution' (Advances in Chemistry Series, No. 117), American Chemical Society, New York, 1973, p. 88.

[357] P. M. Collins, D. Gardiner, and W. G. Overend, *Carbohydrate Res.*, 1974, **32**, 203.

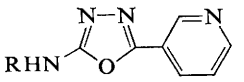
10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines and Related Compounds

Interesting studies of the anomeric and reverse anomeric effects in pentopyranosylamine derivatives have shown that the anomeric effect is strongest when the nitrogen atom bears a formal negative charge; the anomeric effect decreases with substitution at C-1 by the following groups in the order: $\bar{N}-\overset{+}{P}Ph_3$ > OAc > N_3 > $NHCOCF_3$ > $NHCOC_6H_4OMe$-*p* ≈ $NHCOC_6H_4NO_2$-*p* > NH_2 ≈ NHAc ≈ $\overset{+}{N}HPPh_3$ > imidazolium salt > pyridinium salt.[358] 2-Acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine and a number of more complex derivatives have been isolated from the urine of a patient with aspartylglycosaminuria, an inborn error of metabolism.[359] In continuing their investigations on the synthesis of glycopeptides, Jeanloz's group has reported the preparation of peptides containing 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine[360] and of di-*N*-acetylisochitobiose-L-asparagine.[361] Standard syntheses of 6-(2-amino-2-deoxy-β-D-glucopyranosylamino)- and 6-(β-D-glucopyranosylamino)-purine derivatives[362] and of a series of *N*-acetyl-*N*-aryl-α-L-arabinopyranosylamines[363] have been reported. 2,3,4,6-Tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate has been converted into the glycosylamine derivative (165), which exhibited anti-viral activity,[364] and standard procedures have been employed in syntheses of 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (166), isolated from *Lathyrus odoratus* seedlings,[365] the alkaloid caseiniroedine (167), isolated from the Mexican fruit 'Zapote Blanco',[366] and the β-*N*-glycosides of D-glucose and D-ribose with carbazole.[367] *S*-Glycosides of 1-phenyl-5-thiotetrazole re-arranged to the *N*-glycosides (168) on treatment with mercuric bromide.[368]

The yields of glycosylamines resulting from the condensation of aldoses with primary aromatic amines were improved by adding molecular sieves to remove the water produced.[56a]

[358] H. Paulsen, Z. Györgydeák, and M. Friedmann, *Chem. Ber.*, 1974, **107**, 1590.
[359] R. J. Pollitt and K. M. Pretty, *Biochem. J.*, 1974, **141**, 141.
[360] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **32**, 37.
[361] E. Walker and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **32**, 145.
[362] S. Fukatsu, A. Sawa, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1974, **47**, 917.
[363] Z. Smiatacz, *Carbohydrate Res.*, 1974, **34**, 380.
[364] W. Wieniawski, C. Gmernicka-Haftek, M. Korbecki, and E. Walczak, *Acta Polon. Pharm.*, 1973, **30**, 255.
[365] L. Van Rompuy, F. Lambein, R. De Gussem, and R. Van Parijs, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 199.
[366] R. P. Panzica and L. B. Townsend, *J. Amer. Chem. Soc.*, 1973, **95**, 8737.
[367] V. S. Martynov, T. Ya. Filipenko, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1974, **10**, 1117.
[368] G. Wagner, G. Valz, B. Dietzsch, and G. Fischer, *Pharmazie*, 1974, **29**, 90.



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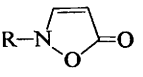

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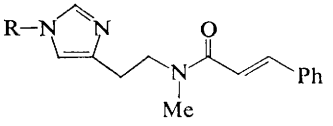
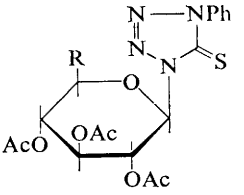


(167)

R = β-D-glucopyranosyl



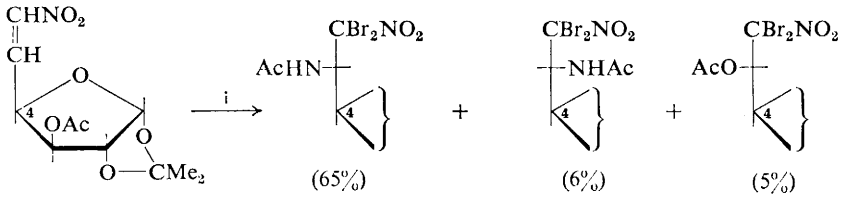
(168) R = H or CH_2OAc

The kinetics of the Amadori rearrangement of D-glucopyranosylamines derived from primary aromatic amines have been investigated.[369]

The formation of glycosylamine-linked disaccharides from uronic acids and amino-sugars is referred to in Chapter 3.

Nitro-sugars

Baer and his co-workers have prepared four isomeric methyl 3,6-dideoxy-3-nitro-α-L-hexopyranosides and have studied the formation of nitro-olefins from mono- and di-acetates thereof.[370] Baer's group has also reported the use of *N*-bromoacetamide with carbohydrates for the conversion of nitro-olefins into *vic*-diamino-sugars by way of acetamido–nitro-derivatives (see Scheme 55); the stereoselectivity of the addition may differ from that obtained with ammonia.[371–373]



(65%) (6%) (5%)

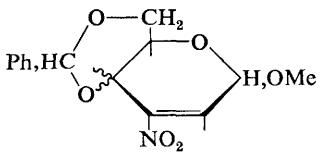
Reagent: i, MeCONHBr

Scheme 55

The major products (170) (R^1 = NHAc, R^2 = Br) from such nitro-olefins as (169) (α- and β-D-*threo*, β-D-*erythro*) had the acetamido-group located *trans* to the aglycone, with the nitro-group assuming an equatorial orientation; this approach led to efficient syntheses of several 2,3-diamino-2,3-dideoxyhexoses.[373] Both α- and β-linked disaccharides containing nitro-groups have been prepared by condensation of benzylated glycosyl bromides with methyl 4,6-*O*-benzylidene-

[369] S. Kolka and J. Sokolowski, *Roczniki Chem.*, 1974, **48**, 439.
[370] H. H. Baer and C.-W. Chiu, *Canad. J. Chem.*, 1974, **52**, 111.
[371] W. Rank and H. H. Baer, *Tetrahedron Letters*, 1974, 1459.
[372] W. Rank and H. H. Baer, *Carbohydrate Res.*, 1974, **35**, 65.
[373] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1974, **52**, 2257.

3-deoxy-3-nitrohexopyranosides under Koenigs–Knorr conditions.[374] Other Michael additions to (169) (α-*threo*) to give (170) [R^1 = N_3, CN, 7-theophyllinyl, or 9-(2,6-dichloropurinyl); R^2 = H] have been shown to result in the thermodynamically less-stable *manno*-configuration.[375] However, treatment with a more basic reagent such as aqueous ammonia yielded exclusively the *gluco*-isomer



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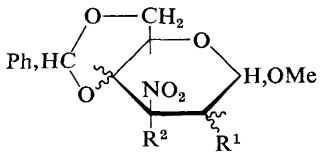
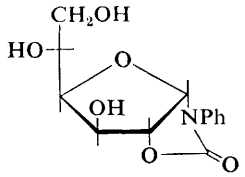


(170)

(170) (R^1 = NH_2, R^2 = H). The addition of nitromethane to D-*xylo*-dialdo-1,4-pentofuranoses took place with high stereoselectivity to give 6-deoxy-6-nitro-D-glucofuranose derivatives.[376] The diazomethane-boron trifluoride reagent can be used to methylate sugars containing a nitro-group [148] (see p. 28).

Heterocyclic Derivatives

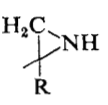
Acid hydrolysis of methyl glycosides containing 2-*O*-phenylcarbamoyl substituents yielded *N*-phenyloxazolone derivatives; *e.g.* methyl 2-*O*-phenylcarbamoyl-α-D-glucopyranoside gave (171)[283] (see also Chapter 6). Aminosugars (*e.g.* 2-amino-2-deoxy-D-glucose) reacted with cyclohexane-1,3-diones to



(171)

produce enamines, which cyclized to give 1,5,6,7-tetrahydroindol-4-one derivatives.[377] Sodium salts of heterocycles have been used in syntheses of 6-deoxy-6-indol-1-yl- and 6-carbazol-9-yl-D-glucoses, by ring-opening of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose,[378] and 5-deoxy-5-(indol-1-yl)uridine, by way of a sulphonate displacement.[378a] Treatment of the 5,6-epimino-L-idofuranose derivative (172) with carbon disulphide yielded the thiazolidine-thione (173), whereas oxazolines (174) were obtained from the reaction with *para*-substituted benzoyl chlorides.[379]

[374] H. H. Baer, J. M. J. Fréchet, and U. Williams, *Canad. J. Chem.*, 1974, **52**, 3337.
[375] T. Sakakibara and R. Sudoh, *J.C.S. Chem. Comm.*, 1974, 69.
[376] V. D. Gusev, T. K. Mitrofanova, O. N. Tolkachev, and R. P. Evstigneeva, *Khim. Khim. Tekhnol., Trudy Yubileinoi Konf., Posvyashch.* 70-*Letiya Inst.* (*Mosk. Inst. Tonkoi Khim. Tekhnol.*), 1970 (publ. 1972), 121.
[377] A. Gómez Sanchez, E. Toledano, and M. Gómez Guillen, *J.C.S. Perkin I*, 1974, 1237.
[378] S. Ya. Mel'nik, E. A. Utkina, M. N. Preobrazhenskaya, and N. N. Suvorov, *Zhur. org. Khim.*, 1974, **10**, 750.
[378a] M. N. Preobrazhenskaya, S. Ya. Mel'nik, E. A. Utkina, E. G. Sokolova, and N. N. Suvorov, *Zhur. org. Khim.*, 1973, **9**, 2207.
[379] M. Iwakawa and J. Yoshimura, *Chem. Letters*, 1974, 519 (*Chem. Abs.*, 1974, **81**, 49 960z).



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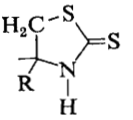

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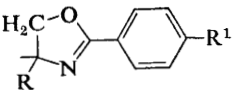
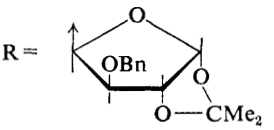
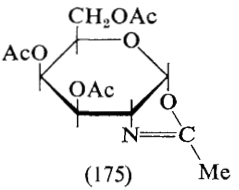


(174) R^1 = NO_2 or OMe



Ammonolysis of peracylated D-fructopyranoses has been found to yield hydroxyalkylated pyrazines and imidazoles, whereas D-fructose gave only 2-amino-2-deoxy-D-glucose.[380] 6-(D-*glycero*-2,3-Dihydroxypropyl)-2-(D-*threo*-trihydroxypropyl)pyrazine has been identified as one of the products of ammonolysis of D-xylose tetranicotinate.[381]

The oxazoline (175) has been used as a glycosylating agent in the synthesis of β-glycosides of 2-acetamido-2-deoxy-D-galactopyranose in condensations with *N*-benzyloxycarbonyl-L-serine benzyl ester, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose.[382]



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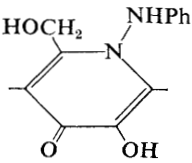

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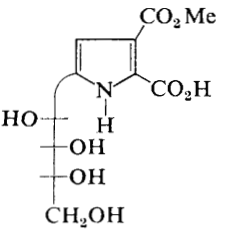

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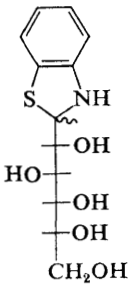

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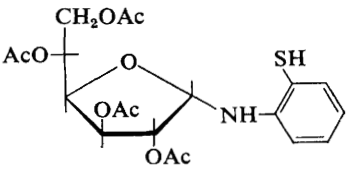
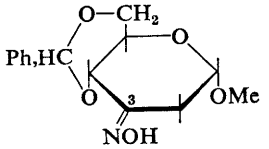


(179)

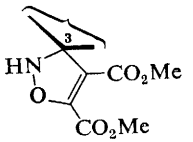
[380] M. C. Teglia and R. A. Cadenas, *Anales Asoc. quim. argentina*, 1973, **61**, 153.
[381] E. A. Forlano, J. O. Deferrari, and R. A. Cadenas, *Carbohydrate Res.*, 1973, **31**, 405.
[382] J.-C. Jacquinet, S. E. Zurabyan, and A. Ya. Khorlin, *Carbohydrate Res.*, 1974, **32**, 137.

In addition to products identified earlier, the reaction of kojic acid with phenylhydrazine has yielded the pyridinone (176).[383] 2-Amino-2-deoxy-D-glucose reacted with dimethyl acetylenedicarboxylate to give a 3-aminofumarate derivative, which cyclized to the pyrrole derivative (177) on heating in water.[384] Treatment of the benzothiazoline derivative (178) with mercuric acetate in methanol afforded a chelate, which, after acetylation and demercuration (with hydrogen sulphide in the absence of air), gave the rearranged glycosamine (179), which regenerated (178) on deacetylation.[385]

The glycosidulose oxime (180) reacted with dimethyl acetylenedicarboxylate to give the spiro-derivative (181) as a minor product, unlike cyclohexanone oxime which did not yield an analogous cyclic adduct.[386]



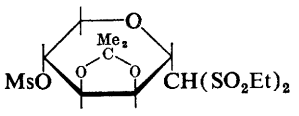
(180)



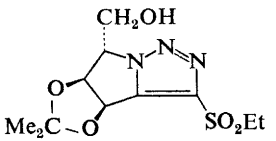
(181)

An investigation, which corrects some results previously reported (J. N. BeMiller and R. K. Mann, *Carbohydrate Res.*, 1966, **2**, 70), has shown that maltose and cellobiose 1-phenylflavazoles undergo acid-catalysed hydrolysis by the same mechanism as do simple glycosides.[387]

A saccharide bis-(1,3,4-oxadiazole) has been prepared by the dehydrative cyclization of an acetylated galactaric acid bis(benzoylhydrazide),[388] and details of a novel reaction of sodium azide in DMF with the sulphonate (182) to give the triazole (183) have appeared.[389]



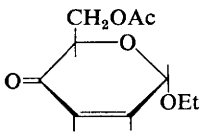
(182)



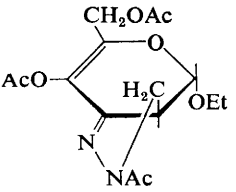
(183)

Acetylation of the product resulting from treatment of the glycenosidulose (184) with diazomethane yielded the pyranopyrazoline (185) possessing mild anti-thrombotic properties.[390] L-*erythro*-Biopterin (187) has been synthesized from 5-deoxy-L-arabinose by way of the mono-oxime (186), as shown in Scheme 56.[391] D-*threo*-Hexo-2,5-diulose and D-*threo*-hexo-2,5-diulosonic acid, which are

[383] E. S. H. El Ashry, *Carbohydrate Res.*, 1974, **33**, 178.
[384] A. Gómez Sanchez, M. Gómez Guillen, E. Pando Ramos, and A. Cert Ventula, *Carbohydrate Res.*, 1974, **35**, 39.
[385] D. S. Boolieris, R. J. Ferrier, and L. A. Branda, *Carbohydrate Res.*, 1974, **35**, 131.
[386] P. M. Collins, D. Gardiner, and W. G. Overend, *Carbohydrate Res.*, 1974, **35**, 251.
[387] J. N. BeMiller, D. R. Smith, V. K. Ghanta, T. O. Lyles, and E. R. Doyle, *Carbohydrate Res.*, 1974, **35**, 255.
[388] M. A. M. Shaban and M. A. U. Nassr, *Carbohydrate Res.*, 1974, **36**, C12.
[389] A. Farrington and L. Hough, *Carbohydrate Res.*, 1974, **38**, 107.
[390] R. M. Srivastava, B. J. Carthy, and B. Fraser-Reid, *Tetrahedron Letters*, 1974, 2175.
[391] E. C. Taylor and P. A. Jacobi, *J. Amer. Chem. Soc.*, 1974, **96**, 6781.



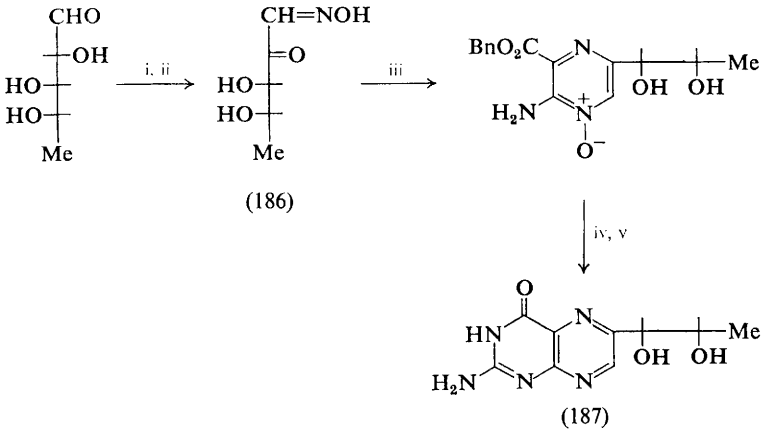
(184)



(185)

available on a large scale by bacterial oxidation of the corresponding sugars at C-5, reacted with hydrazine to give the 4(1*H*)-pyridazinone derivatives (188).[392]

The oxime (189) obtained from methyl 4,6-*O*-benzylidene-α-D-*erythro*-hexopyranosid-2,3-diulose 3-phenylhydrazone (163) (see Chapter 9) cyclized to give

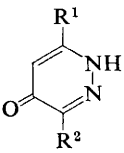


(186)

(187)

Reagents: i, $Cu(OAc)_2$; ii, $Me_2C{=}NOH$; iii, $H_2NCH(CN)CO_2Bn$; iv, $(NH_2)_2C{=}NH$; v, $Na_2S_2O_4$

Scheme 56



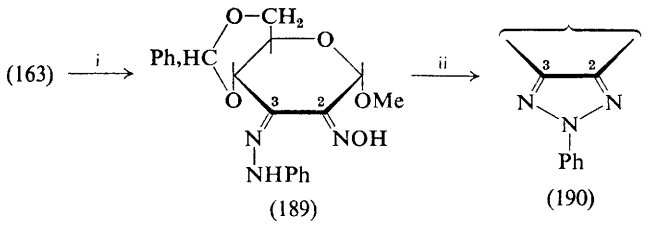
(188)

$R^1 = R^2 = CH_2OH$
$R^1 = CH_2OH$; $R^2 = CO_2H$
$R^1 = CO_2H$; $R^2 = CH_2OH$

the phenylosotriazole (190) on treatment with toluene-*p*-sulphonyl chloride in pyridine (Scheme 57). Sequential treatment of the 2-azidoglycosid-3-ulose (191) with triethylamine and *o*-phenylenediamine furnished the quinoxaline derivative (192) by way of an unstable α-imino-ketone, as shown in Scheme 58.[357]

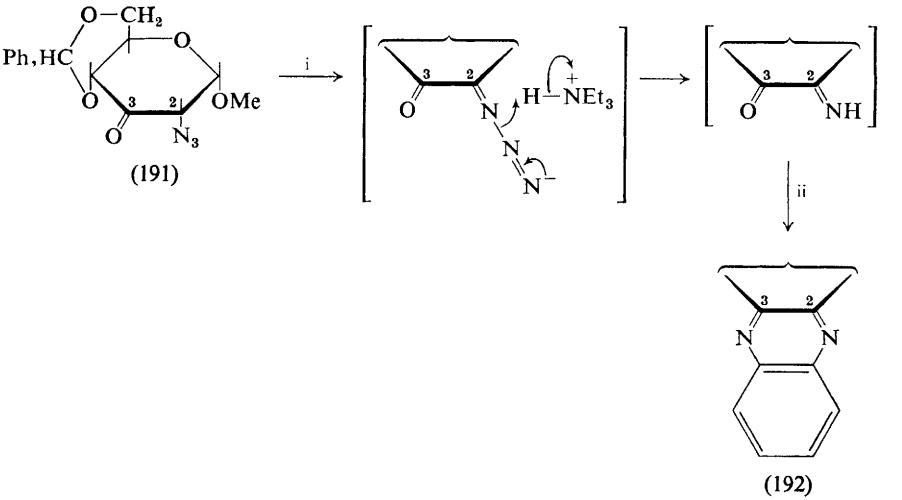
The equilibria established between 2-acylamino-2-deoxy-D-glucopyranosyl halides and the corresponding oxazolinium halides have been found to depend on the polarity of the solvent; whereas only the pyranosyl halide was detected in

[392] K. Imada and K. Asano, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1691.



Reagents: i, NH_2OH; ii, TsCl–py

Scheme 57

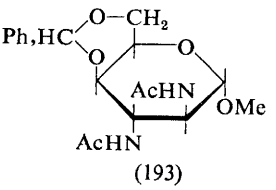


Reagents: i, Et_3N; ii, o-$C_6H_4(NH_2)_2$

Scheme 58

non-polar solvents, a reversible equilibrium was established in polar, aprotic media.[393]

The synthesis and reactions of the anomeric methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-D-gulo- and -talo-pyranosides have been reported.[394] Azidolysis of the α-D-*talo*-epimine was used in a synthesis of a derivative (193) of 2,3-diacetamido-2,3-dideoxy-D-idose.



[393] P. Stoeckl, H. Hoeng, and H. Weidmann, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1974, **1**, 169.

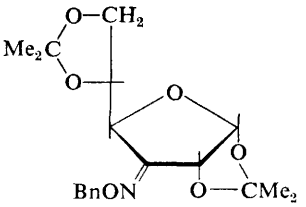
[394] R. D. Guthrie and J. A. Liebmann, *J.C.S. Perkin I*, 1974, 650.

The formation of heterocyclic compounds from dicarbonyl sugars is referred to in Chapter 15.

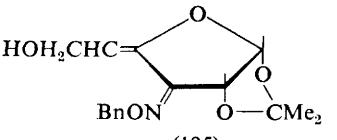
Miscellaneous Compounds

Glycosyl azides having the substituents at C-1 and C-2 *trans*-related have been prepared by treating acetylated pyranoses with trimethylsilyl azide and a Lewis acid catalyst; the orientation of the azido-group can be predicted using the octant rule.[395] Whereas u.v. irradiation of hexopyranosyl azides resulted in loss of nitrogen and bond cleavage to give the corresponding pentose, pentosyl azides gave intermediates that reverted to the starting materials on storage in the dark.[39] A study of azide displacements on a series of representative sulphonates is referred to in Chapter 6, and the ring-opening reactions of 2,3-anhydrohexose derivatives by azide ions are considered in Chapter 4.

2-Deoxy-3,5-di-*O*-*p*-tolyl-α- and -β-D-*erythro*-pentofuranosyl cyanides have been prepared by standard methods,[396] and 2,3,5-tri-*O*-benzyl-D-ribofuranosyl cyanides have been used for the synthesis of 'homonucleosides'.[397] Syntheses of guanidino-derivatives of sugars, from the corresponding amino-sugars,[398] and of *O*-benzyl-oximes, from the corresponding glycosuloses,[399] have been reported; the compound (194) gave the unsaturated derivative (195) when treated with sodium



(194)

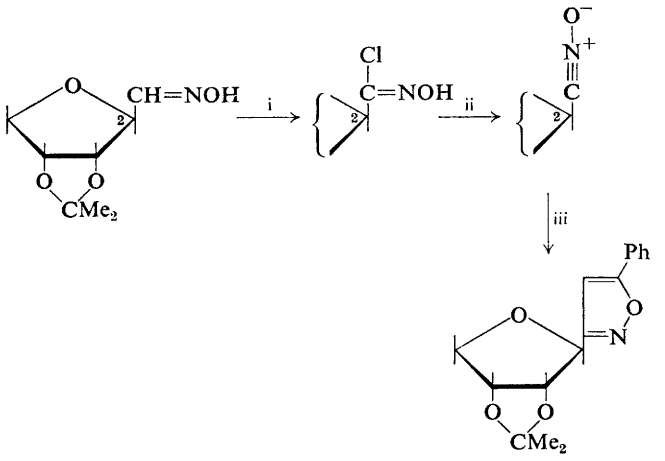


(195)

borohydride. The oxime derived from 2,5-anhydro-3,4-*O*-isopropylidene-*aldehydo*-D-ribose has been converted into a nitrile oxide, which reacted with phenylacetylene to give, *inter alia*, the isoxazole (196) (Scheme 59).[400]

Several papers have dealt with *N*-methylnitrones prepared from sugars containing a free carbonyl group by treatment with *N*-methylhydroxylamine. In each case, only the *anti*(*Z*)-isomer was obtained [*e.g.* (197; R = Me)].[401] Reaction of (197; R = H) in sequence with hydrogen cyanide, hydroxylamine, and water afforded 5-amino-5-deoxyhexuronic acid derivatives (198),[402] whilst reduction of a branched-chain nitro-sugar yielded a cyclic nitrone by way of a transient hydroxylamino-sugar (see Scheme 60).[403] The photochemistry of a number of nitrones has been investigated.[402]

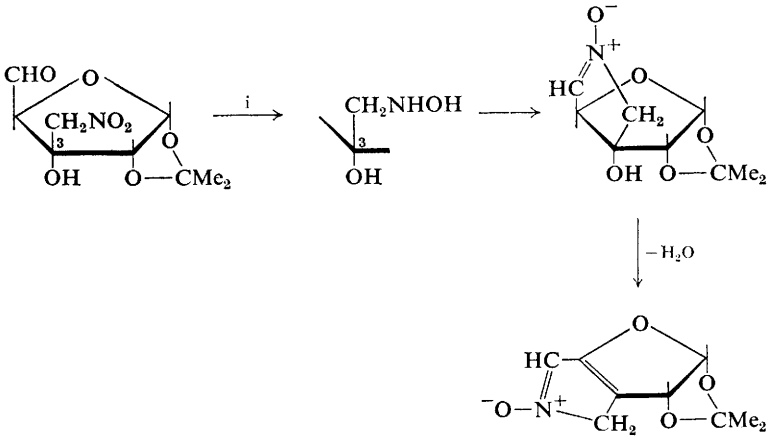
[395] H. Paulsen, Z. Györgydeák, and M. Friedmann, *Chem. Ber.*, 1974, **107**, 1568.
[396] A. Kolb, T. H. Dinh, and J. Igolen, *Bull. Soc. chim. France*, 1973, 3447.
[397] M. W. Winkley, *Carbohydrate Res.*, 1973, **31**, 245.
[398] J. Yoshimura, T. Sekiya, and Y. Ogura, *Bull. Chem. Soc. Japan*, 1974, **47**, 1219.
[399] J. Plenkiewicz, W. A. Szarek, P. A. Sipos, and M. K. Phiobs, *Synthesis*, 1974, 56.
[400] J. M. J. Tronchet and F. Perret, *Carbohydrate Res.*, 1974, **38**, 169.
[401] J. M. J. Tronchet and E. Mihaly, *Carbohydrate Res.*, 1973, **31**, 159.
[402] H. Paulsen and M. Budzis, *Chem. Ber.*, 1974, **107**, 2009.
[403] H. Paulsen and M. Budzis, *Chem. Ber.*, 1974, **107**, 1998.



(196)

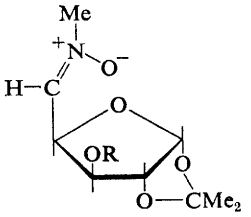
Reagents: i, Cl_2; ii, Et_3N; iii, PhC≡CH

Scheme 59

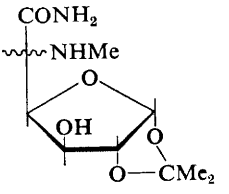


Reagent: i, Zn

Scheme 60

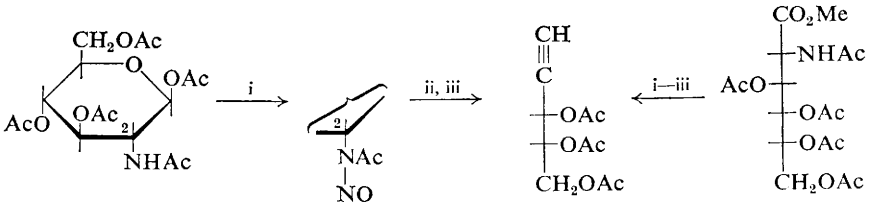


(197)



(198)

Dinitrogen tetraoxide has been used to convert 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose into the *N*-nitroso-derivative, which yielded an acetylenic sugar on treatment with base[404] (see Scheme 61). Other acetylenic



Reagents: i, N_2O_4; ii, KOH; iii, Ac_2O–py

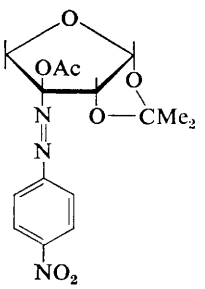
Scheme 61

sugars have been similarly prepared, and methyl 3,4,5,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitroso)acetamido-D-gluconate was degraded with base to an acetylenic sugar with loss of carbon dioxide.[404a]

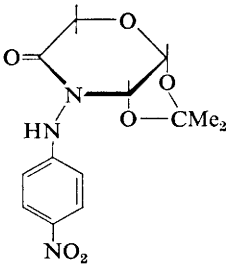
Gentiobiose octa-acetate reacted with ammonia to give 1,1-bis(acetamido)-1-deoxy-6-*O*-β-D-glucopyranosyl-D-glucitol and *N*-acetyl-6-*O*-β-D-glucopyranosyl-β-D-glucofuranosylamine.[405]

The catalytic effect of anions on the Maillard reaction of sugars with glycine[406] and a number of the reactions of 4,5,6-tri-*O*-benzoyl-1-diazo-1,3-dideoxy-D-*erythro*-hexulose have been examined.[407] *N*-Glycosyl-*N'*-alkylurea derivatives gave novel nitroso-derivatives on nitrosation in formic acid.[408]

Treatment of 1,2-*O*-isopropylidene-α-D-*erythro*-tetros-3-ulose *p*-nitrophenylhydrazone with lead tetra-acetate yielded the *gem*-azo-acetate (199), which rearranged with ring-expansion to give the *N*-amino-lactam (200) on treatment with potassium t-butoxide.[409]



(199)



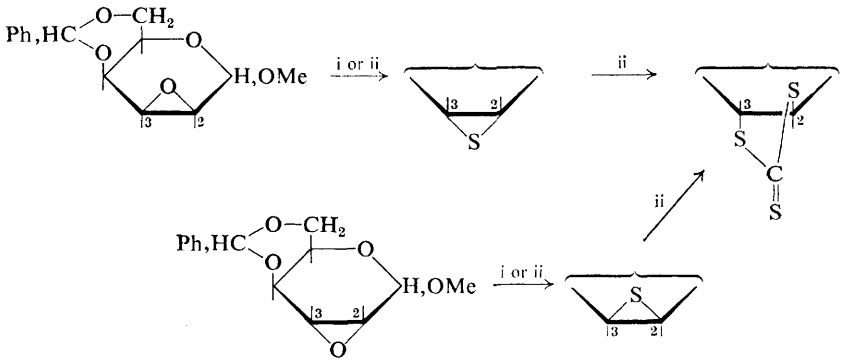
(200)

[404] D. Horton and W. Loh, *Carbohydrate Res.*, 1974, **36**, 121.
[404a] D. Horton and W. Loh, *Carbohydrate Res.*, 1974, **38**, 189.
[405] A. B. Zanlungo, J. O. Deferrari, M. E. Gelpi, and R. A. Cadenas, *Carbohydrate Res.*, 1974, **35**, 33.
[406] F. O. Bobbio, P. A. Bobbio, and L. M. V. Trevisan, *Lebensm.-Wiss. Technol.*, 1973, **6**, 215 (*Chem. Abs.*, 1974, **80**, 83 421p).
[407] M. L. Wolfrom, N. Kashimura, and D. Horton, *Carbohydrate Res.*, 1974, **36**, 211.
[408] J. L. Montero and J.-L. Imbach, *Compt. rend.*, 1974, **279**, *C*, 809.
[409] J. M. J. Tronchet, F. Rachidzadeh, and J. Tronchet, *Helv. Chim. Acta*, 1974, **57**, 65.

11
Thio- and Seleno-sugars

Thio-sugars

Episulphides have been prepared from methyl 2,3- and 3,4-anhydrohexopyranosides by reaction with potassium thiocyanate or thiourea (see, *e.g.*, Scheme 62); the yields of the episulphides varied, and other products, including

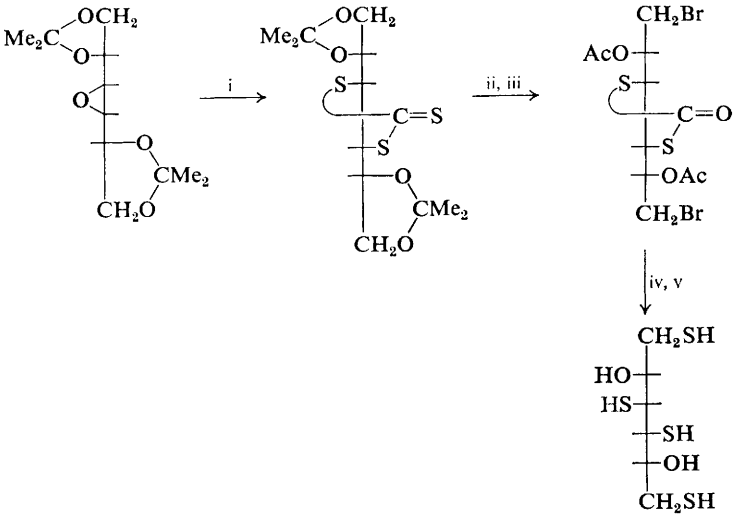


Reagents: i, KCNS or $(NH_2)_2C{=}S$; ii, MeOC=SSNa

Scheme 62

unsaturated glycosides, predominated in the reactions of β-glycosides.[410] Prolonged treatment with potassium thiocyanate resulted in partial stereomutation of the thiiran ring, whereas desulphurization occurred if the reaction with thiourea was prolonged. The aforementioned 2,3- and 3,4-anhydro-sugars and the corresponding episulphides were converted into *trans*-trithiocarbonates on treatment with sodium methyl xanthate, with inversion of configuration at the site of the initial attack (see Scheme 62).[411] Other products included unsaturated derivatives, hydroxy-thiols (formed by hydrolytic opening of the oxiran ring), and related *S*-methyl derivatives. The epoxides and episulphides selected for investigation were of the *cis*-decalin type, having conformational mobility sufficient to allow the formation of *trans*-fused, five-membered rings in the products or the intermediates. A trithiocarbonate has been obtained as an intermediate in a synthesis of 1,3,4,6-tetrathio-D-mannitol (Scheme 63).[412]

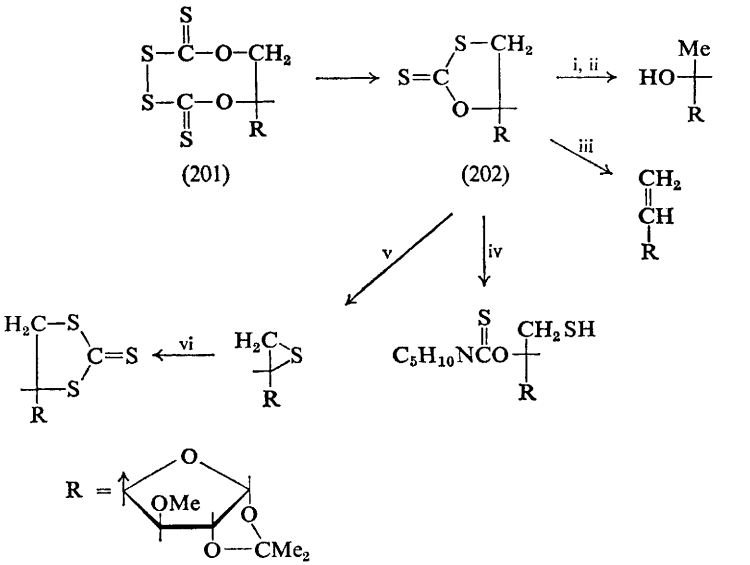
[410] M. V. Jesudason and L. N. Owen, *J.C.S. Perkin I*, 1974, 2019.
[411] M. V. Jesudason and L. N. Owen, *J.C.S. Perkin I*, 1974, 2024.
[412] G. E. McCasland, A. B. Zanlungo, and L. J. Durham, *J. Org. Chem.*, 1974, **39**, 1462.



Reagents: i, MeOC=SSK; ii, $KMnO_4$; iii, HBr–HOAc; iv, AcSK; v, $LiAlH_4$

Scheme 63

Xanthation of 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose furnished an unstable 5,6-dithiobis(thioformate) derivative (201), which decomposed to give the 5,6-*OS*-dithiocarbonate (202); the compound (202) was used in syntheses of unsaturated sugars and thio- and deoxy-sugars (see Scheme 64).[413] Similar

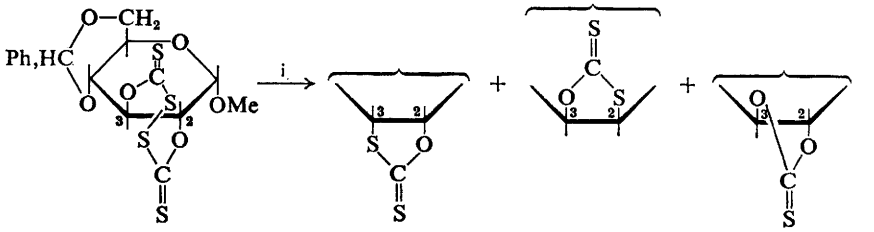


Reagents: i, $NaBH_4$; ii, Raney Ni; iii, inactive Ni; iv, piperidine; v, NaOEt; vi, MeOC=SSNa

Scheme 64

[413] B. S. Shasha and W. M. Doane, *Carbohydrate Res.*, 1974, **34**, 370.

xanthation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside afforded the *trans*-2,3-cyclic ester (203) (which decomposed on pyrolysis or upon treatment with base in DMSO to give *OS*-dithiocarbonates, with inversion at the site of thiolation) and a 2,3-thionocarbonate (Scheme 65). The dithiobis(thioformate)



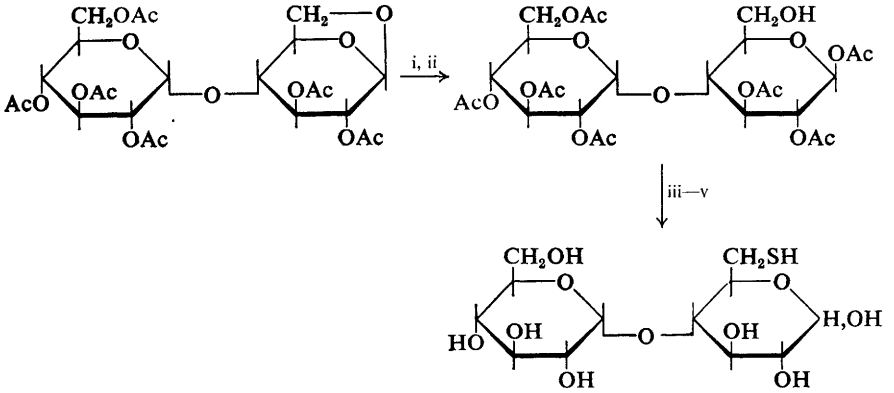
(203)

Reagent: i, heat or dicyclohexylamine–DMSO

Scheme 65

of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside decomposed only to the thionocarbonate and the diol.[414]

Thiomaltose derivatives containing a sulphur atom at either C-1 or C-6 of the reducing-sugar residue, including the 1,6-anhydro-analogue (6-thiomaltosan), have been prepared; the 6-thio-derivative was obtained by way of maltosan hexa-acetate, as shown in Scheme 66.[415] Nucleoside derivatives of 5-thio-D-arabinofuranose are referred to in Chapter 21.



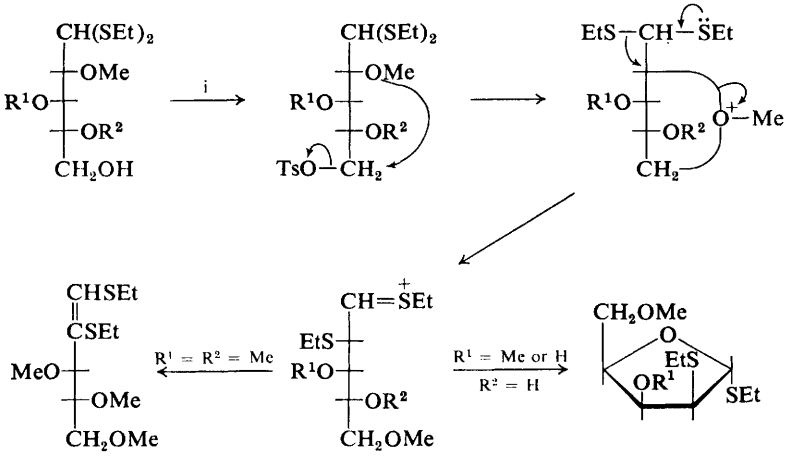
Reagents: i, $TiBr_4$; ii, $Hg(OAc)_2$; iii, TsCl–py; iv, AcSK; v, MeONa–MeOH

Scheme 66

One of the ethylthio-groups migrated from C-1 to C-2 on toluene-*p*-sulphonylation of 2-mono-, 2,3-di-, and 2,3,4-tri-*O*-methyl-D-xylose diethyl dithioacetals.[192] The various products of these reactions were considered to result from participation by the methoxy-group at C-2 in the displacement of the 5-sulphonate

[414] B. S. Shasha, D. Trimnell, and W. M. Doane, *Carbohydrate Res.*, 1974, **32**, 349.
[415] M. Mori, M. Haga, and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1331.

initially formed and subsequent migration of an ethylthio-group, as illustrated in Scheme 67. Ethanethiolysis of the penta-acetates of D-fructose and L-sorbose in the presence of zinc chloride has been shown to yield significant proportions of



Reagent: i, TsCl–py

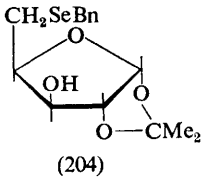
Scheme 67

3,4,5,6-tetra-*O*-acetyl-1-*S*-ethyl-1-thio-D-fructose and -L-sorbose diethyl dithioacetals, respectively, in addition to the expected dithioacetals.[416]

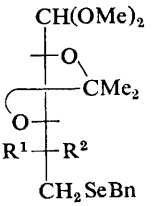
Other thio-sugars are mentioned in Chapter 12, while Chapter 3 contains references to 1-thioglycosides and glycosyl sulphones.

Seleno-sugars

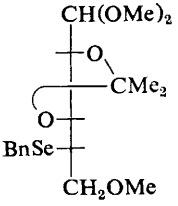
Methanolysis of 5-*Se*-benzyl-1,2-*O*-isopropylidene-5-seleno-α-D-xylofuranose (204) is claimed to give the acyclic acetals (205) and (206).[417] The first oxides of seleno-sugars have been obtained from *Se*-alkylated 5-seleno-D-xylo- and -ribofuranose derivatives by oxidation with periodate, hydrogen peroxide, or ozone.[418]



(204)



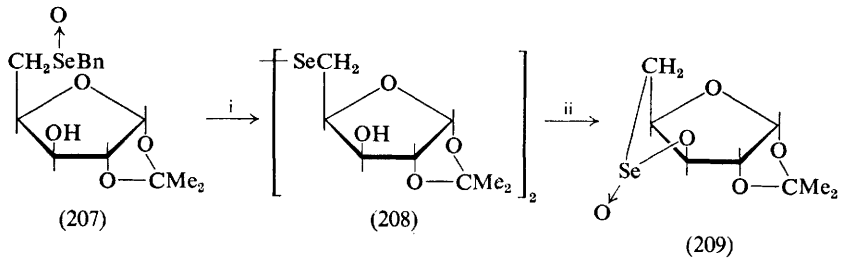
(205) R^1 = H(OMe); R^2 = OMe(H)



(206)

[416] G. S. Bethell and R. J. Ferrier, *Carbohydrate Res.*, 1974, **34**, 194.
[417] T. Van Es and J. Rabelo, *Carbohydrate Res.*, 1974, **36**, 408.
[418] J. Rabelo and T. Van Es, *Carbohydrate Res.*, 1974, **32**, 175.

In one case, ^1H n.m.r. spectroscopy indicated the presence of two isomeric selenoxides. All the selenoxides decomposed on warming, regenerating the parent selenide; in the case of the benzyl selenoxide (207), thermal degradation gave benzaldehyde and a diselenide (208), oxidation of which furnished a compound tentatively identified as the internal selenic ester (209) (Scheme 68).



(207) (208) (209)

Reagents: i, heat; ii, 30% H_2O_2

Scheme 68

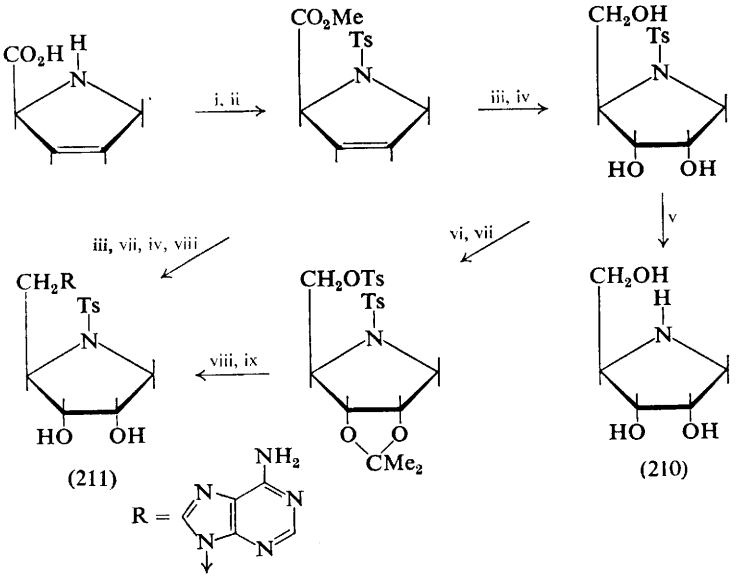
12

Derivatives with Nitrogen, Sulphur, or Phosphorus in the Sugar Ring

Nitrogen Derivatives

The synthesis and a study of 2,4-diamino-2,4-dideoxy-D-galactose in solution have been mentioned [349] in Chapter 8.

The preparation [419] of 1-amino-1,4-anhydro-1-deoxy-DL-ribitol (210) from dehydro-DL-proline and conversions [420] yielding the 'reversed' nucleoside (211) are shown in Scheme 69. Oxidation of nojirimycin (5-amino-5-deoxy-D-gluco-

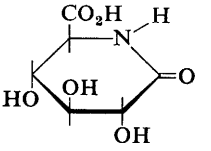


Reagents: i, TsCl–NaOH; ii, CH_2N_2; iii, $LiBH_4$; iv, OsO_4; v, NH_3–Na; vi, $Me_2C(OMe)_2$; vii, TsCl–py; viii, $C_5H_4N_5Na$; ix, H^+

Scheme 69

pyranose) with sodium hypoiodite and then with oxygen in the presence of a platinum catalyst gave the acid (212), which is a potent inhibitor of β-glucuronidase.[421]

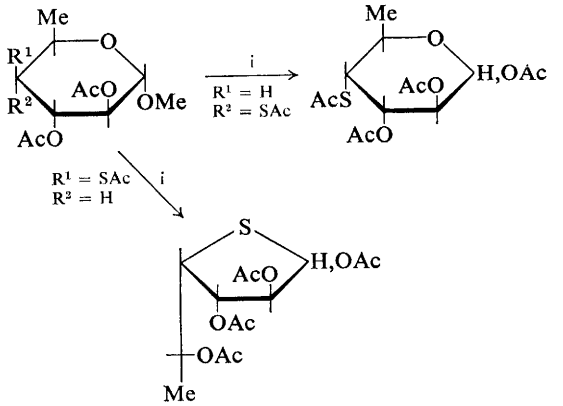
[419] V. Nair and R. H. Walsh, *Carbohydrate Res.*, 1974, **36**, 131.
[420] V. Nair and R. H. Walsh, *J. Org. Chem.*, 1974, **39**, 3045.
[421] T. Tsuruoka, T. Niwa, T. Shomura, T. Matsuno, N. Itoh, S. Inoue, and T. Niida, *Meiji Seika Kenkyu Nempo*, 1973, 80 (*Chem. Abs.*, 1974, **81**, 120 901p).



(212)

Sulphur Derivatives

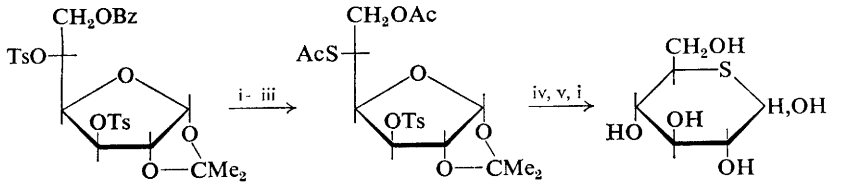
Syntheses of 6-deoxy-4-thio-D-altrose and -idose have been reported by routes involving displacements with thiocyanate ions on methyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-methylsulphonyl-α-D-ido- and -altro-pyranosides, respectively.[422]



Reagent: i, Ac_2O–H_2SO_4

Scheme 70

^1H N.m.r. spectroscopy showed that 6-deoxy-4-thio-D-idose preferentially adopts the α-furanoid form in aqueous acetone, whereas the *altro*-compound exists as an equilibrium mixture of three components. Differences were also noted between the acetolyses of methyl 2,3-di-*O*-acetyl-4-*S*-acetyl-6-deoxy-4-thio-α-D-altro- and -ido-pyranosides (see Scheme 70). A variation of an earlier synthesis of 5-thio-D-glucose (Vol. 3, p. 103) is shown in Scheme 71.[422a]



Reagents: i, NaOMe–MeOH; ii, $(H_2N)_2C{=}S$; iii, AcOH–Ac_2O–AcOK; iv, NH_3–Na; v, AcOH–Ac_2O–H_2SO_4

Scheme 71

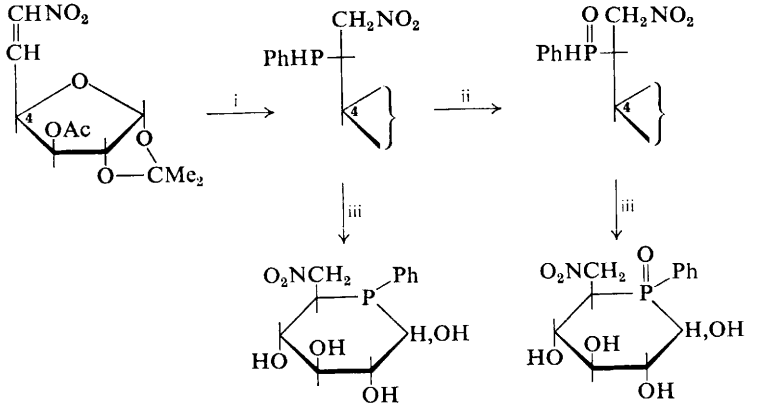
[422] B. Gross and F.-X. Oriez, *Carbohydrate Res.*, 1974, **36**, 385.

[422a] M. M. A. Abd El-Rahman and R. L. Whistler, *Org. Prep. Proc. Internat.*, 1973, **5**, 245 (*Chem. Abs.*, 1973, **79**, 146 749d).

The mass spectrometry of derivatives of 5-thio-D-glucose and 4-thio-D-arabinose is discussed in Chapter 24.

Phosphorus Derivatives

Inokawa's group has reported the first syntheses of sugars containing either a tervalent or a quinquevalent phosphorus atom in a six-membered, hemiacetal ring (see Scheme 72); the structure of each product was confirmed by ^{1}H n.m.r. spectroscopy.[423]



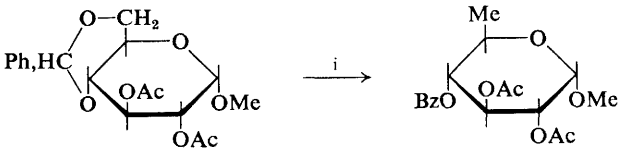
Reagents: i, $PhPH_2$; ii, H_2O_2–MeOH; iii, H^+

Scheme 72

[423] H. Takayanagi, M. Yamashita, K. Seo, H. Yoshida, T. Ogata, and S. Inokawa, *Carbohydrate Res.*, 1974, **38**, C19.

13
Deoxy-sugars

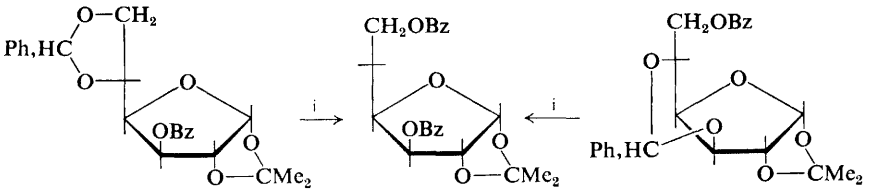
A new synthesis of deoxy-sugars follows from the observation that cyclic benzylidene acetals were converted into deoxy-sugar benzoates on heating with di-t-butyl peroxide in a radical-induced reaction (see, *e.g.*, Scheme 73); although



Reagent: i, $Bu^t_2O_2$

Scheme 73

only the 6-deoxy-sugar was formed in this instance, both 4- and 6-deoxy-sugar derivatives were obtained when methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactopyranoside was treated similarly.[424] Several applications of the reagent, particularly in the D-glucofuranose series (Scheme 74), were described.



Reagent: i, $Bu^t_2O_2$

Scheme 74

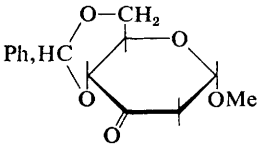
Hydrogenolysis of benzoylated glycosyl bromides over palladized charcoal in the presence of triethylamine gave substantial proportions of benzoylated 2-deoxy-sugars, in addition to benzoylated 1,5-anhydroalditols, when the substituents at C-1 and C-2 were *cis* related.[425] The *trans*-isomers afforded only benzoylated 1,5-anhydroalditols on similar reduction.

Variose, a dideoxy-sugar component of variamycin, has been identified as 2,6-dideoxy-4-*O*-methyl-D-*ribo*-hexose.[426]

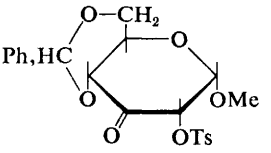
[424] L. M. Jeppesen, I. Lundt, and C. Pedersen, *Acta Chem. Scand.*, 1973, **27**, 3579.
[425] S. Jacobsen and C. Pedersen, *Acta Chem. Scand.*, 1973, **27**, 3111.
[426] G. B. Lokshin, Yu. V. Zhdanovich, A. D. Kuzovkov, and V. I. Sheichenko, *Khim. prirod. Soedinenii*, 1973, **9**, 418.

Tritiated 2-, 3-, 4-, and 6-deoxy-D-glucose derivatives, specifically labelled in the deoxy-groups, and related deuteriated 2- and 3-deoxy-derivatives have been prepared by appropriate hydrogenolysis of the corresponding iodo-sugars.[427, 427a]

The photolysis of α-keto-sulphonates in the presence of an organic base has been shown to result in reductive removal of the ester group; *e.g.*, the 2-deoxy-derivative (213) was obtained from (214) in approximately 30% yield.[428] A



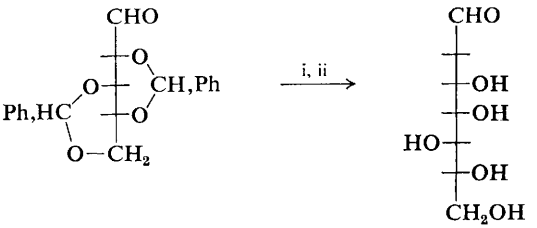
(213)



(214)

radical mechanism, involving an enol intermediate, was suggested to account for the formation of the products. A related reduction is noted in Chapter 4.

2-Deoxyheptoses have been prepared by treating suitably protected *aldehydo*-pentoses with ethyl oxalacetate (see Scheme 75).[429]



Reagents: i, $EtO_2CCOCH_2CO_2Et$; ii, H^+

Scheme 75

Hydrogenation of methyl 2,3-anhydro-α- and -β-D-lyxofuranosides over a Raney nickel catalyst furnished only the 3-deoxyglycosides as products; these were converted into 3-deoxy-2,5-di-*O*-*p*-nitrobenzoyl-α-D-*threo*-pentofuranosyl bromide, a glycosylating agent used in the preparation of 3′-deoxynucleosides.[430]

New syntheses of D-rhamnose and 6-deoxy-D-glucose (D-quinovose) have been effected by the reaction sequences shown in Scheme 76.[431] 6-Deoxy-L-glucose (L-quinovose) was similarly obtained by epimerization of the corresponding L-rhamnose derivative at C-2 during acetolysis.

Derivatives of 6-deoxyhexopyranosides have been prepared by reduction of 6-deoxyhex-5-enopyranosides derived by pyrolysis of 6-dithiocarbonates.[432]

[427] V. Soukupová, K. Vereš, J. Beneš, and M. Černý, *Radiopharm. Label. Compounds Proc Symp. New Develop. Radiopharm. Label. Compounds*, 1973, **2**, 231 (*Chem. Abs.*, 1974, **81**, 91 825j).

[427a] V. Soukupová, M. Černý, and K. Vereš, *Radiochem. Radioanalyt. Letters*, 1974, **18**, 107 (*Chem. Abs.*, 1974, **81**, 120 877k).

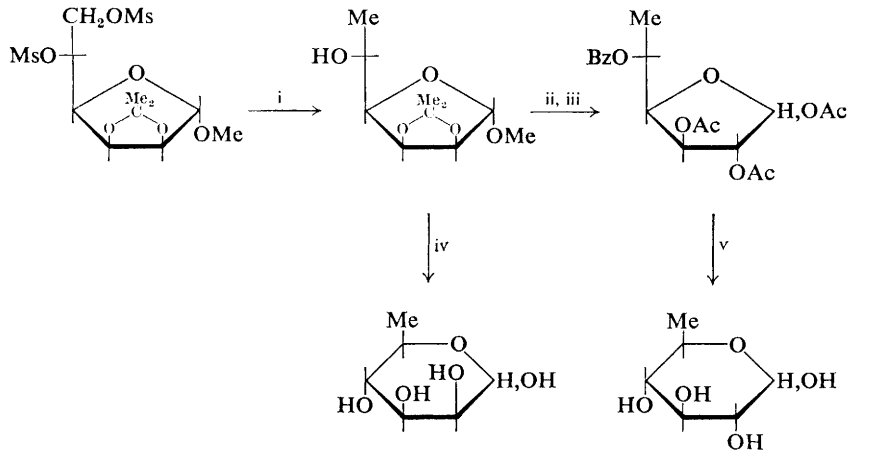
[428] W. A. Szarek and A. Dmytraczenko, *Synthesis*, 1974, 579.

[429] H. Zinner and J. Weber, *J. prakt. Chem.*, 1974, **316**, 13.

[430] H. S. El Khadem, T. D. Audichya, and M. J. Withee, *Carbohydrate Res.*, 1974, **33**, 329.

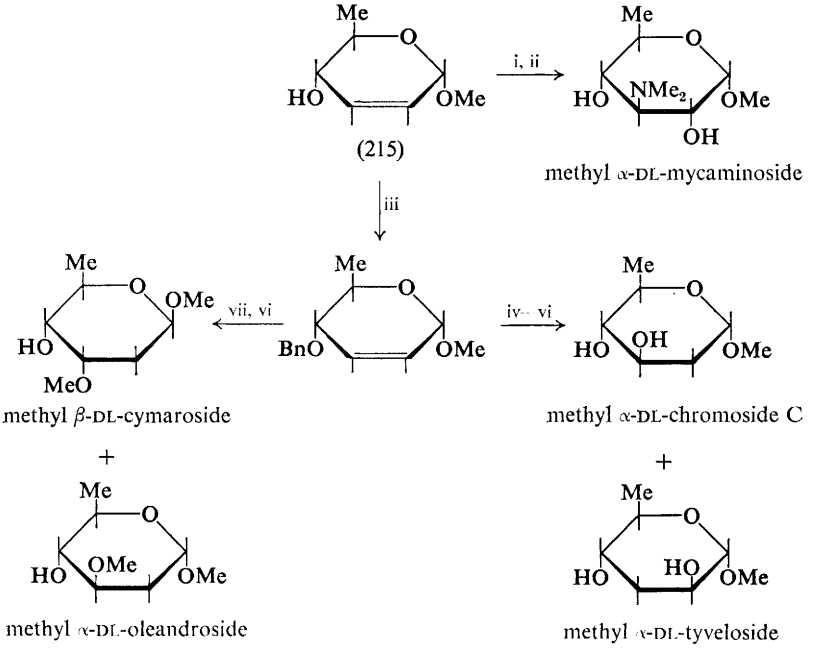
[431] L. M. Lerner, *Carbohydrate Res.*, 1974, **36**, 392.

[432] G. Descotes, A. Faure, and J. C. Martin, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1974, **1**, 133.



Reagents: i, $LiAlH_4$; ii, BzCl–py; iii, $AcOH–Ac_2O–H_2SO_4$; iv, H^+; v, MeONa

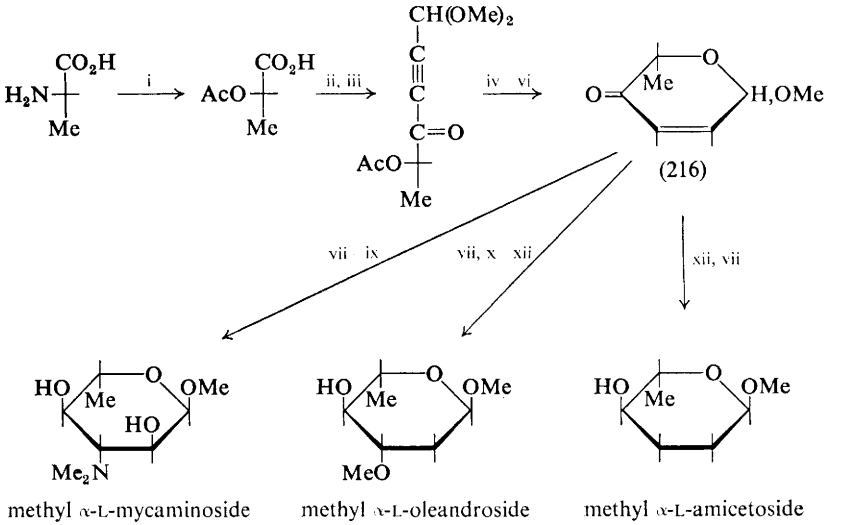
Scheme 76



Reagents: i, $PhCO_3H$; ii, Me_2NH; iii, BnCl; iv, $Hg(OAc)_2$; v, $NaBH_4$; vi, H_2–Pd; vii, $MeOH–H^+$

Scheme 77

The glycosides of several antibiotic sugars (as racemates) have been synthesized from methyl 2,3,6-trideoxy-α-DL-*erythro*-hex-2-enopyranoside (215) by the reactions outlined in Scheme 77.[433] The enone (216), prepared from L-alanine, was utilized in closely related work to obtain the α-glycosides of L-mycaminose, L-oleandrose, and L-amicetose (Scheme 78).[434] 4,6-Dideoxyhexose derivatives



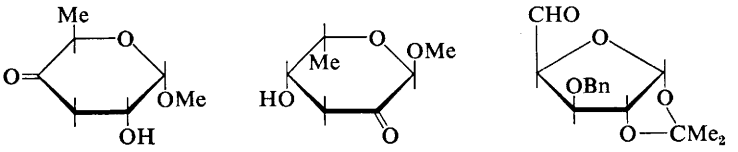
methyl α-L-mycaminoside methyl α-L-oleandroside methyl α-L-amicetoside

Reagents: i, HNO_2–AcOH; ii, $SOCl_2$; iii, $(MeO)_2CHC{\equiv}CMgBr$; iv, H_2–Pd–$BaSO_4$; v, HO^-; vi, H^+; vii, $LiAlH_4$; viii, *m*-$ClC_6H_4CO_3H$; ix, Me_2NH; x, BnCl; xi, MeOH–H^+; xii, H_2–Pd–C

Scheme 78

related to aldgarose and derivatives of pentopyranine A [a (2,3-dideoxypentosyl)-nucleoside] are noted in Chapters 15 and 20, respectively.

The deoxy-derivatives (217) and (218) were obtained during investigations of the ring-opening reactions of oxirans derived from hexopyranosiduloses (see



also Scheme 42).[307] The addition of ethylmagnesium bromide to the 5-aldehydo-sugar (219) has led to syntheses of 6,7-dideoxy-D-*gluco*- and -L-*ido*-heptoses,[435] and 4,4′-di- and 4,4′,6,6′-tetradeoxy-analogues of αα-trehalose have been synthesized by way of reductive dehalogenation of di-iodo- and tetrachloro-disaccharides.[436]

[433] S. Yasuda and T. Matsumoto, *Tetrahedron*, 1973, **29**, 4087.
[434] K. Koga, S.-I. Yamada, M. Yoh, and T. Mizoguchi, *Carbohydrate Res.*, 1974, **36**, C9.
[435] H. Walls, jun. and D. E. Kiely, *Carbohydrate Res.*, 1973, **31**, 397.
[436] G. G. Birch, C.-K. Lee, and A. C. Richardson, *Carbohydrate Res.*, 1974, **36**, 97.

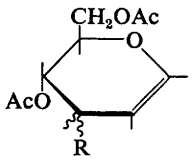
14
Unsaturated Derivatives

Glycals

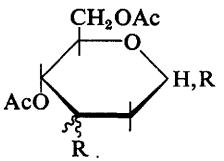
A study of the conformations of acetylated glycals by ^{1}H n.m.r. spectroscopy is referred to in Chapter 23.

3,4,6-Tri-*O*-acetyl-D-allal has been prepared by standard procedures, and traditional aspects of its chemistry have been examined.[437]

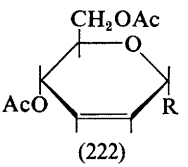
Methoxyhalogenation of 3,4-di-*O*-acetyl-D-xylal has been reported to give methyl 3,4-di-*O*-acetyl-2-deoxy-2-halogeno-α-D-lyxo- and -β-D-xylo-pyranosides; the latter derivatives were transformed into methyl 2,3-anhydro-β-D-lyxopyranoside on treatment with sodium methoxide.[438] 2,2-Dichloro-3,4-di-*O*-acetyl-α-D-*threo*-pentopyranosyl chloride was also prepared from the glycal. The epimeric glycals (220) reacted with benzotriazole in the presence of trifluoroacetic acid to give the adducts (221) and the 2,3-unsaturated derivative (222) arising from sigmatropic rearrangement.[439]



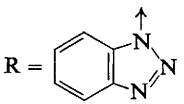
(220)



(221)



(222)



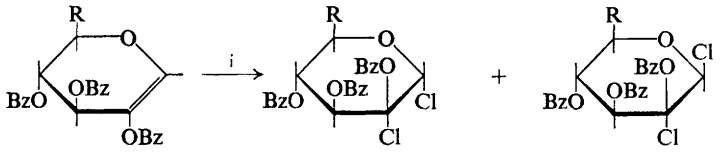
The addition of nitrosyl chloride to 3,4-di-*O*-acetyl-L-rhamnal is referred to in Chapter 10 and the photochemical addition of methanol to a glycal enone is mentioned in Chapter 16; the photochemical addition of lactonitrile to 3,4,6-tri-*O*-acetyl-D-glucal is referred to below.

Further aspects of the chemistry of 2-hydroxyglycals have been published during the past year. 2,3,4,6-Tetra-*O*-acetyl-2-hydroxy-D-allal has been prepared and additions to the double bond were examined; *e.g.* catalytic hydrogenation

[437] M. Haga and S. Tejima, *Carbohydrate Res.*, 1974, **34**, 214.
[438] T. Van Es, *J. S. African Chem. Inst.*, 1973, **26**, 152.
[439] G. García-Muñoz, F. G. De Las Heras, R. Madroñero, and M. Stud, *Anales de Quim.*, 1973, **69**, 1335 (*Chem. Abs.*, 1974, **80**, 96 283v).

over a palladium catalyst afforded 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-altritol exclusively.[437]

The chlorination of various benzoylated 2-hydroxyglycals yielded adducts (Scheme 79) whose stereochemistries were assigned by n.m.r. spectroscopy (for

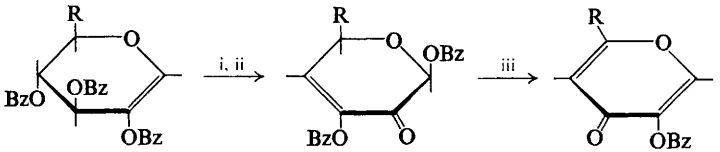


R = CH_2OBz, Me, or H

Reagent: i, Cl_2–C_6H_6

Scheme 79

C-1) and c.d. (for C-2).[440] Anomerization of the β-chlorides with titanium tetrachloride gave α-chlorides only when R = Me or CH_2OBz, whereas a mixture containing equal proportions of each anomer was obtained in the pentose series (R = H). Enones were formed when the adducts were treated with base, and these could be converted into 4-pyrones (Scheme 80).[441] The related pyrones

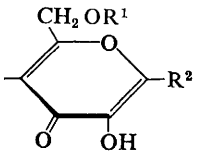


R = CH_2OBz, Me, or H

Reagents: i, Cl_2; ii, $NaHCO_3$–H_2O; iii, AcOH–AcONa

Scheme 80

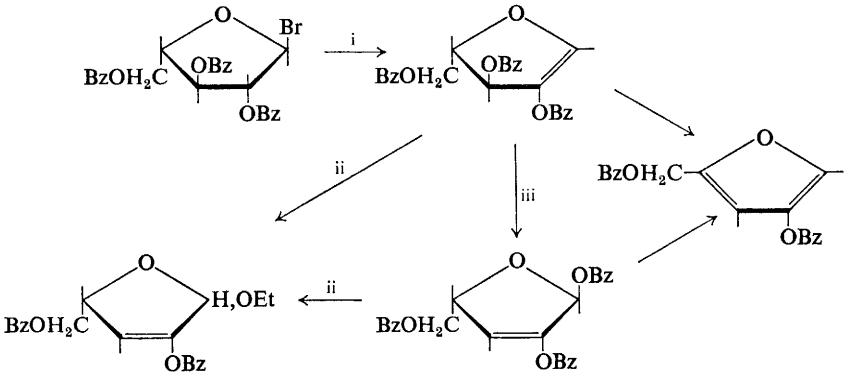
(223) and (224) furnished the bromides (225) and (226), respectively, on treatment with NBS, but a reaction did not occur when the hydroxy-group at C-2 was substituted.[442]



(223) R^1 = Ac, R^2 = H
(224) R^1 = Bz, R^2 = H
(225) R^1 = Ac, R^2 = Br
(226) R^1 = Bz, R^2 = Br

[440] F. W. Lichtenthaler and E. Fischer, *Angew. Chem. Internat. Edn.*, 1974, **13**, 548.
[441] E. Fischer and F. W. Lichtenthaler, *Angew. Chem. Internat. Edn.*, 1974, **13**, 546.
[442] L. Tolentino and J. Kagan, *J. Org. Chem.*, 1974, **39**, 2308.

The first specific syntheses of furanoid 2-hydroxyglycals have been reported; these glycals were shown to be significantly more susceptible to sigmatropic rearrangement and elimination than are the pyranoid analogues (Scheme 81).[443] Compounds in this series were judged to be too reactive to serve as satisfactory glycosylating agents.



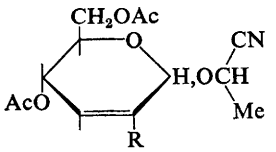
Reagents: i, DBU; ii, EtOH; iii, room temperature

Scheme 81

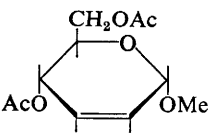
Derivatives of 3-cyanomethyl-3-deoxyglycals have been encountered during the synthesis of branched-chain sugar nucleosides (see Chapter 15),[444, 445] and the first syntheses of 1′,2′-unsaturated nucleosides are noted in Chapter 21.

Other Unsaturated Compounds

3,4,6-Tri-*O*-acetyl-D-glucal and the 2-acetoxy-derivative thereof furnished the unsaturated glycosides (227) and (228), respectively, on irradiation in the presence of lactonitrile.[446] It would be interesting to know which chromophore is involved and whether acid-catalysed reactions are also involved in the photolytic addition of lactonitrile to (229), which also yielded (227). The reactions did not take



(227) R = H
(228) R = OAc

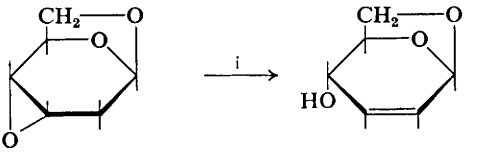


(229)

place in the dark, but the same products were obtained when pre-irradiated lactonitrile reacted with the glycals, suggesting that radical processes are operative.

[443] R. J. Ferrier and J. R. Hurford, *Carbohydrate Res.*, 1974, **38**, 125.
[444] A. Rosenthal and C. M. Richards, *Carbohydrate Res.*, 1974, **32**, 53.
[445] A. Rosenthal and C. M. Richards, *Carbohydrate Res.*, 1974, **32**, 67.
[446] K. Matsuura, K. Senna, Y. Araki, and Y. Ishido, *Bull. Chem. Soc. Japan*, 1974, **47**, 1197.

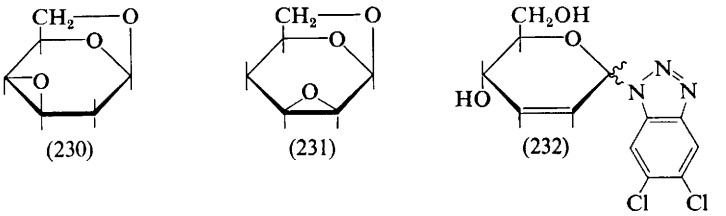
Further work (*cf.* Vol. 4, p. 6 and Vol. 5, pp. 8, 33) on achiral 1,6:3,4-dianhydro-2-deoxyhexopyranoses and 1,6:2,3-dianhydro-4-deoxyhexopyranoses has shown that they are converted into allylic alcohols with strong bases (see, *e.g.*, Scheme 82).[447] Whereas the *lyxo*-compounds (230) and (231) underwent com-



Reagent: i, BuLi

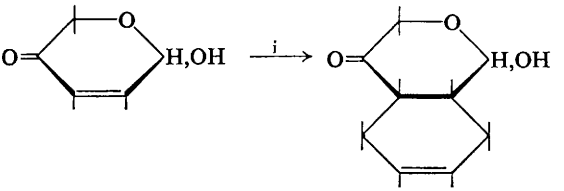
Scheme 82

petitive opening of the oxiran ring with butyl-lithium, the allylic alcohols could be obtained using lithium diethylamine.



Epoxidation of the β-nucleoside (232; β-anomer) with hydrogen peroxide in benzonitrile gave a low yield of the corresponding 2,3-anhydro-D-allopyranoside, whereas the α-anomer afforded the isomeric 2,3-anhydro-D-mannopyranoside.[448]

Now that enones have become readily accessible derivatives of carbohydrates, it is of interest to note that 2-hydroxy-5-oxo-5,6-dihydro-2*H*-pyran is an effective dieneophile (Scheme 83).[449] Benzoylation of D-glucono-1,5-lactone with an excess of benzoyl chloride in pyridine afforded the unsaturated derivative (233).[450]



Reagent: i, $CH_2{=}CHCH{=}CH_2$

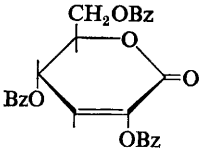
Scheme 83

[447] K. Ranganayakulu, U. P. Singh, T. P. Murray, and R. K. Brown, *Canad. J. Chem.*, 1974, **52**, 988.

[448] G. García-Muñoz, F. G. De La Heras, R. Madroñero, and M. Stud, *Anales de Quim.*, 1974, **70**, 283 (*Chem. Abs.*, 1974, **81**, 4194m).

[449] G. Jones, *Tetrahedron Letters*, 1974, 2231.

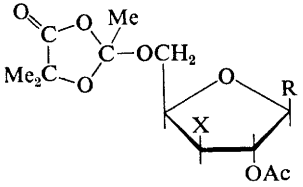
[450] R. M. de Lederkremer, M. I. Litter, and L. F. Sala, *Carbohydrate Res.*, 1974, **36**, 185.



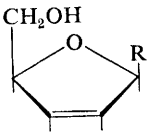
(233)

New addition reactions of 2,3-unsaturated 3-nitrohexopyranosides are described in Chapter 10, and the mass spectrometry of enones is referred to in Chapter 24.

The furanoid olefin (66) has been used in the synthesis of aldoses from non-carbohydrate precursors,[184] and a related report describes an improved route to 2′,3′-unsaturated furanosyl nucleosides by way of the reactions of 2-acetoxyisobutyryl halides.[451] Thus, the nucleoside orthoester (234) reacted with chromous acetate and ethylenediamine in ethanol at −78 °C to give the unsaturated derivative (235), whereas the olefin (236) was readily obtained when (234) was



(234)



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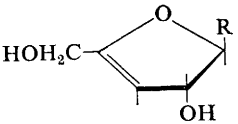

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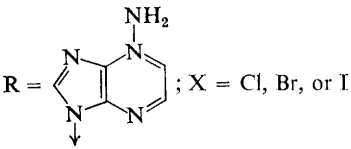
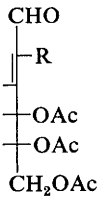


treated with DBU. Other examples are given of the application of this approach to the synthesis of nucleosides containing unsaturated sugars.

Two reports have described syntheses of enals. Acetolysis of isopropyl 2-acetamido-2-deoxy-αβ-D-glucopyranoside furnished the *trans*-olefin (237),



(237) R = NHAc
(238) R = H
(239) R = Br

[451] T. C. Jain, I. D. Jenkins, A. F. Russell, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 30.

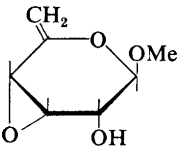
which isomerized to the *cis*-form on u.v. irradiation.[452] The unsaturated, acyclic derivatives (238) and (239) resulted from treatment of 1,3,4,5,6-penta-*O*-acetyl-1,2-*O*-isopropylidene-D-glucose with hydrogen bromide in acetic acid (see Scheme 27).[219]

The base-catalysed degradation of such compounds as 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitroso)acetamido-β-D-glucopyranose to five-carbon, acetylenic sugar derivatives has been mentioned already (Chapter 10),[404, 404a] and other unsaturated acyclic derivatives are referred to in Chapter 5.

The conformations of methyl 3,4-dideoxy-DL-glyc-3-enopyranosides have been studied by ^{1}H n.m.r. spectroscopy (see Chapter 23), and syntheses of (*E*)-3,4-dideoxy-*erythro*-, (*Z*)-3,4-dideoxy-D-*threo*-, and (*E*)-3,4-dideoxy-D-*threo*-hex-3-enitols have been described.[453] The action of potassium selenocyanate on 1,2:5,6-di-*O*-isopropylidene-D-mannitol 3,4-bis(toluene-*p*-sulphonate) was shown to give the (*E*)-D-*threo*-compound in low yield and only traces of the (*Z*)-D-*threo*-isomer.

The formation of unsaturated sugar derivatives by elimination reactions on hexuronic acids has been reviewed,[454] and the antibiotic sisomycin, containing an unsaturated sugar component, is mentioned in Chapter 20.

The pyrolysis of methyl 2,3,4-tri-*O*-acetylhexopyranoside 6-dithiocarbonates has yielded 5,6-unsaturated derivatives, which afforded 6-deoxyglycosides on hydrogenation over Raney nickel.[432] Related unsaturated sugars, obtained by treating 6-iodoglycosides with silver fluoride, have been converted into the oxiran derivatives (240)—(242).[280] 6-Deoxyhex-5-enopyranosides with a good



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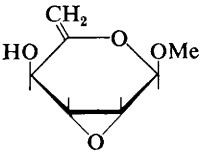

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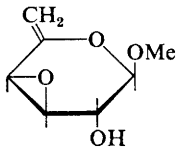
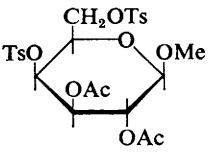


(242)



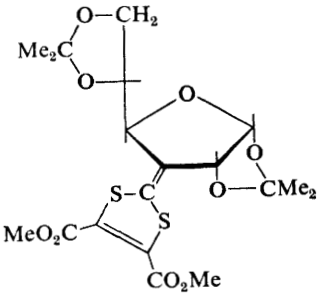
(243)

leaving group at C-4 were found to undergo attack by iodide ion at C-6 by an S_N2' process, and successive treatments of the disulphonate (243) with sodium iodide, silver fluoride, and base resulted in the formation of allomaltol (see Scheme 40).

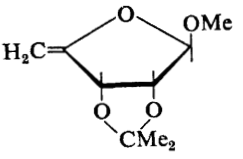
[452] E. W. Thomas, *Carbohydrate Res.*, 1974, **33**, 175.
[453] G. O. Aspinall, N. W. H. Cheetham, J. Furdova, and S. C. Tam, *Carbohydrate Res.*, 1974, **36**, 257.
[454] J. Kiss, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 229.

The exocyclic olefin (244) has been used in the synthesis of branched-chain sugars (Chapter 15), and u.v. irradiation of the unsaturated sugar (245) in the presence of lactonitrile furnished the adduct (246).[446] Chain elongation of derivatives of *aldehydo*-D-arabinose, to give $\alpha\beta$-unsaturated esters (247), has been accomplished by means of the Knoevenagel reaction.[455]

C-Glycosides containing unsaturated linkages in the aglycone are discussed in Chapter 3.



(244)



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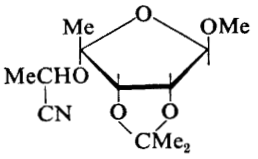

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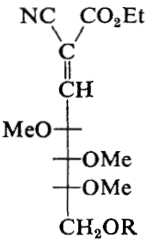


(247) R = Ac, Tr, Bz, or Bn

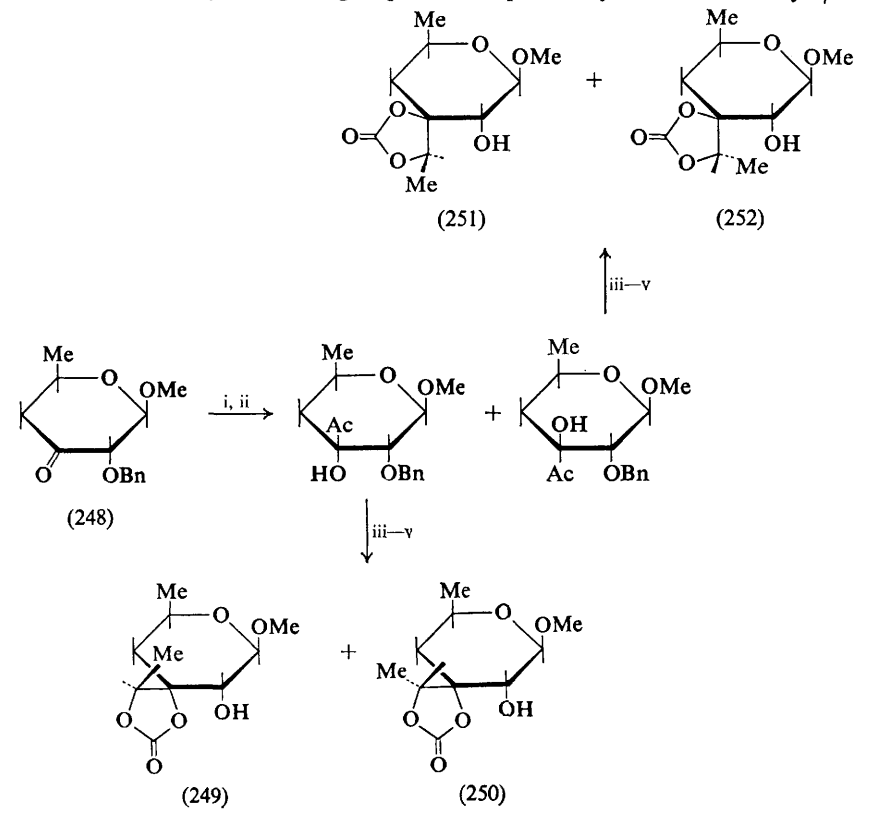
[455] J. Csaszar and V. Bruckner, *Ann. Univ. Sci. Budapest Rolando Eotvos Nominatae, Sect. Chim.*, 1973, 87 (*Chem. Abs.*, 1974, **80**, 83 437y).

15
Branched-chain Sugars

A review on the natural occurrence and significance of branched-chain sugars, including some aspects of their chemical synthesis, has appeared.[456]

Compounds with an $R^1—C—OR^2$ Branch

There has been considerable interest in the synthesis of naturally occurring branched-chain sugars. Two groups have reported syntheses of methyl β-D-

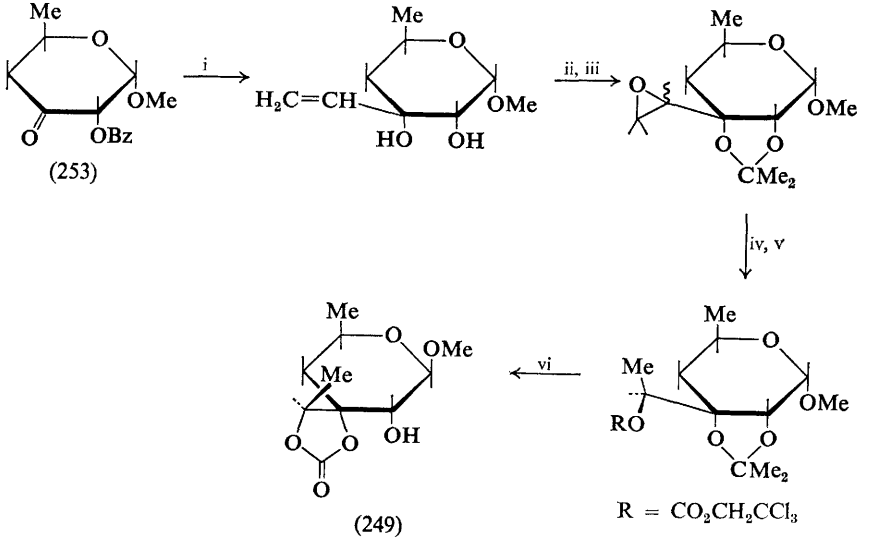


Reagents: i, ; ii, $HgCl_2$–HgO; iii, $NaBH_4$; iv, $(PhO)_2CO$; v, H_2–Pd

Scheme 84

[456] H. Paulsen, *Stärke*, 1973, **25**, 389.

aldgaroside (249), which was thereby shown to have the (*S*)-configuration in the chain branch. Paulsen's group treated the β-glycosid-3-ulose (248) with 2-lithio-2-methyl-1,3-dithian to give isomeric 3-*C*-acetyl derivatives, which were converted into the four possible stereoisomers (249)—(252) as outlined in Scheme 84; the isomer (249) was shown to be indistinguishable from methyl β-D-aldgaroside, obtained by methanolysis of aldgamycin E.[457] A more stereoselective synthesis was achieved by Brimacombe's group, who treated the α-glycosid-3-ulose (253) with vinylmagnesium bromide and then epoxidized the acetalated 3-*C*-vinyl derivative (Scheme 85). The major oxiran obtained was subsequently trans-



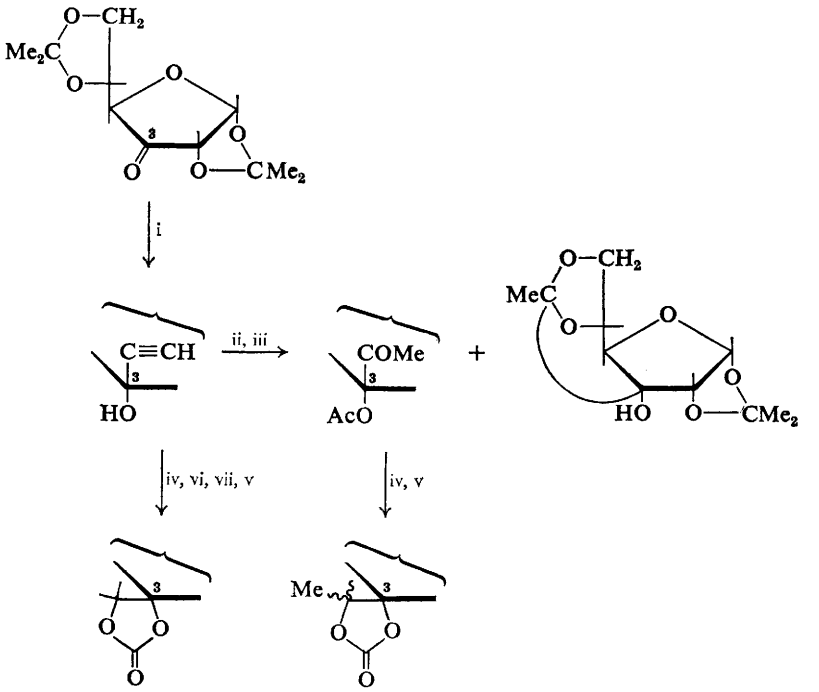
Reagents: i, CH_2=CHMgBr; ii, $Me_2C(OMe)_2$–TsOH; iii, *m*-$ClC_6H_4CO_3H$; iv, $LiAlH_4$; v, $Cl_3CCH_2O \cdot COCl$; vi, MeOH–H^+

Scheme 85

formed into methyl β-D-aldgaroside (249).[458] In another study, branched-chain sugars related to D-aldgarose were synthesized from 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose by the sequence of reactions shown in Scheme 86.[459]

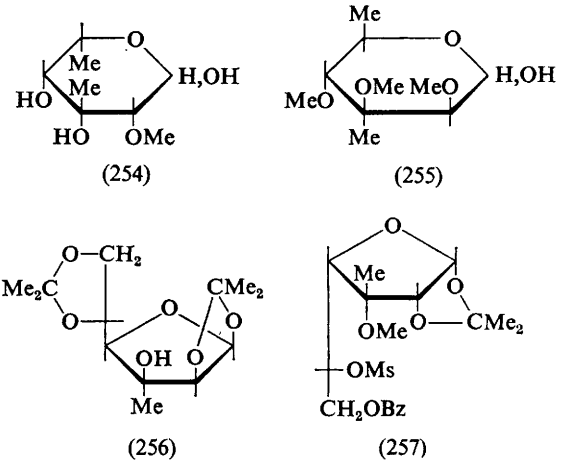
Two closely related syntheses have established that L-vinelose is 6-deoxy-3-*C*-methyl-2-*O*-methyl-L-talose (254);[460, 461] both syntheses relied on appropriate modifications of 1,2:5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (Vol. 7, p. 116). Brimacombe's group has also reported a synthesis of D-nogalose (6-deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-D-mannopyranose) (255) in a seventeen-step sequence from methyl α-D-glucopyranoside by way of 1,2:5,6-di-*O*-isopropylidene-3-*C*-methyl-β-D-mannofuranose (256), prepared by a Grignard

[457] H. Paulsen and H. Redlich, *Chem. Ber.*, 1974, **107**, 2992.
[458] J. S. Brimacombe, C. W. Smith, and J. Minshall, *Tetrahedron Letters*, 1974, 2997.
[459] D. C. Baker, D. K. Brown, D. Horton, and R. G. Nickol, *Carbohydrate Res.*, 1974, **32**, 299.
[460] J. S. Brimacombe, S. Mahmood, and A. J. Rollins, *Carbohydrate Res.*, 1974, **38**, C7.
[461] M. Funabashi, S. Yamazaki, and J. Yoshimura, *Tetrahedron Letters*, 1974, 4331.



Reagents: i, HC≡CMgBr; ii, $Hg(OAc)_2$; iii, H_2S; iv, $LiAlH_4$; v, $COCl_2$–py; vi, O_3; vii, $NaBH_4$

Scheme 86



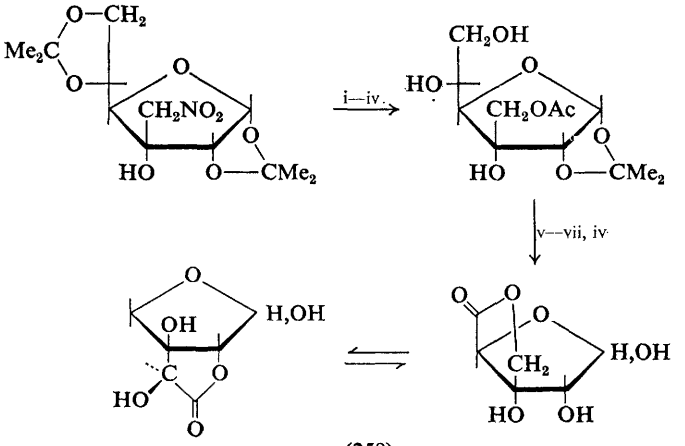
(254) (255)

(256) (257)

reaction on the corresponding glycos-3-ulose.[462] A related approach to L-nogalose, the naturally occurring sugar, broke down when the methanesulphonate (257) failed to yield an epoxide after debenzoylation, presumably for steric reasons.

[462] J. S. Brimacombe and A. J. Rollins, *J.C.S. Perkin I*, 1974, 1568.

Two groups have reported syntheses of 3-*C*-hydroxymethyl-D-riburonic acid, which has been suggested to be the branched-chain uronic acid component of a bilirubin conjugate.[463–464a] However, Kuenzle's group has now shown that the synthetic and natural sugars are not identical. Both syntheses were developed from 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose with introduction

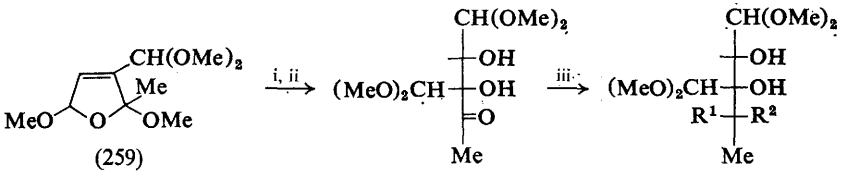


(258)

Reagents: i, $KMnO_4$; ii, $NaBH_4$; iii, Ac_2O–py; iv, H^+; v, $NaIO_4$; vi, HO^-; vii, Ac_2O–DMSO

Scheme 87

of the chain branch by either the nitromethane (Scheme 87)[463] or the methyldithian procedures.[464, 464a] Subsequent transformations afforded 3-*C*-hydroxymethyl-D-riburonic acid as a mixture of the free acid and the lactones (258).



(259)

(260) $R^1 = OH$, $R^2 = H$
(261) $R^1 = H$, $R^2 = OH$

Reagents: i, $KMnO_4$; ii, *aci*-resin; iii, $LiAlH_4$ or $NaBH_4$

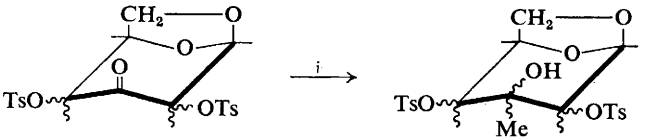
Scheme 88

The racemic streptose derivative (260) and the *ribo*-epimer (261) have been synthesized from the dihydrofuran derivative (259), as outlined in Scheme 88.[465]

The addition of methylmagnesium iodide to a series of 1,6-anhydro-2,4-di-*O*-toluene-*p*-sulphonyl-β-D-hexopyranos-3-uloses has been investigated; the stereo-

[463] W. P. Blackstock, C. C. Kuenzle, and C. N. Eugster, *Helv. Chim. Acta*, 1974, **57**, 1003.
[464] H. Paulsen and W. Stenzel, *Chem. Ber.*, 1974, **107**, 3020.
[464a] H. Paulsen and W. Stenzel, *Tetrahedron Letters*, 1974, 25.
[465] J. Srogl, M. Janda, and J. Stibor, *Coll. Czech. Chem. Comm.*, 1974, **39**, 185.

chemistries of the additions resembled those of reductions with sodium borohydride, with steric effects governing the approach of the reagent accounting for the results obtained (see Scheme 89).[466] The equilibria between the free 3-*C*-

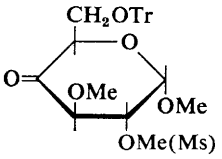


D-*arabino* ⇌ D-*manno*, D-*altro* (1:1)
D-*lyxo* ⇌ D-*talo*
D-*ribo* ⇌ D-*allo*

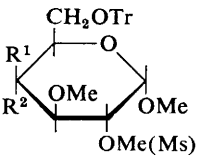
Reagent: i, MeMgI

Scheme 89

methylhexoses and the 1,6-anhydrides thereof, and the formation of borate complexes by the anhydrides (see Chapter 18), were also examined. Stereoselective syntheses of 3- and 5-*C*-methyl-D-glucofuranose derivatives and branched epimers thereof, using standard routes from either *C*-methylene or aldosulose intermediates, have been reported briefly.[467] The D-*xylo*-hexopyranosid-4-ulose derivatives (262) reacted with methyl-lithium to give the 4-*C*-methyl-D-*gluco*-compounds (263), whereas they reacted stereospecifically with methylmagnesium iodide to give the 4-*C*-methyl-D-*galacto*-compounds (264).[468]



(262)



(263) R^1 = Me; R^2 = OH
(264) R^1 = OH; R^2 = Me

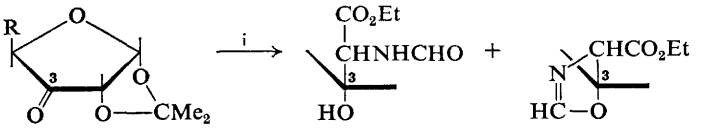
The difference in behaviour of the organometallic reagents was attributed to the formation of a complex between magnesium and O-3 and the carbonyl group of (262), resulting in axial methylation. {Analogous behaviour has already been observed in the reactions of methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose with organo-lithium and -magnesium reagents [A. A. J. Feast, W. G. Overend, and N. R. Williams, *J. Chem. Soc.* (*C*), 1966, 303].}

Details concerning the formation of *C*-cyanomethyl derivatives from the reaction of methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose with acetonitrile (*cf.* Vol. 4, p. 104) have now appeared.[469]

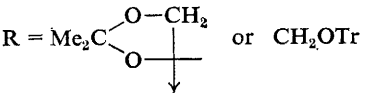
A number of 4-*C*-hydroxymethylated pentofuranose derivatives, including 9-[4-*C*-(hydroxymethyl)-α-L-*threo*-pentofuranosyl]adenine, have been prepared

[466] M. Černý, M. Kollmann, J. Pacák, and M. Buděšínsky, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2507.
[467] M. Funabashi, H. Sato, and J. Yoshimura, *Chem. Letters*, 1974, 803 (*Chem. Abs.*, 1974, **81**, 91 823g).
[468] M. Miljković, M. Gligorijević, T. Satoh, and D. Miljković, *J. Org. Chem.*, 1974, **39**, 1379.
[469] A. Rosenthal and G. Schöllnhamer, *Canad. J. Chem.*, 1974, **52**, 51.

by condensation of formaldehyde with dialdose derivatives (*e.g.* methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdofuranoside).[470] Details of the reaction of ethyl isocyanoacetate with glycosuloses (*cf.* Vol. 5, p. 103) have also been disclosed.[471] In weakly basic medium, simple adducts (265) and the heterocyclic derivative (266) (Scheme 90) were formed, whereas *C*-methylene derivatives



(265) (266)

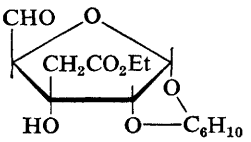


Reagents: i, EtO_2CCH_2NCO–NaCN–EtOH

Scheme 90

[*e.g.* 3-deoxy-3-*C*-ethoxycarbonyl(formylamino)methylene-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose] resulted when the reactions were conducted in the presence of sodium hydride.

The stereochemistry of the product of a Reformatski reaction on 1,2:5,6-di-*O*-cyclohexylidene-α-D-*ribo*-hexofuranos-3-ulose has been determined by standard degradation to the dialdose (267), which exhibited adsorption at 1720 cm^{-1} attributable to a free aldehydo-group; this evidence is indicative of the D-*ribo* configuration, since the isomeric *xylo*-compound would be expected to form a dimeric hemiacetal.[472]



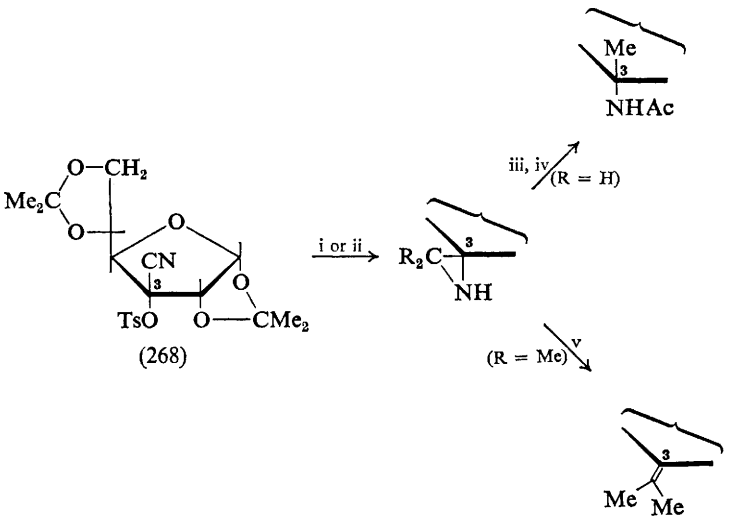
(267)

Reference is made in other chapters to syntheses of dihydrostreptomycin (Chapter 20), branched-chain amino-sugars (Chapter 8), 5′-mono- and 5′,5′-di-*C*-methyl derivatives of adenosine 3′,5′-cyclic phosphate (Chapter 21), and disaccharides incorporating L-mycarose (Chapter 3). The use of $J_{^{13}C-^{1}H}$ values for assigning the configurations of branched-chain sugars is discussed in Chapter 23, and the chiroptical properties of 2-*C*-methylpentono-1,4-lactones are mentioned in Chapter 25. Spectrophotometric determinations of 3-deoxy-2-*C*-hydroxymethylpentonic acids are reported in Chapter 26.

[470] D. L. Leland and M. P. Kotick, *Carbohydrate Res.*, 1974, **38**, C9.
[471] A. J. Brink and A. Jordaan, *Carbohydrate Res.*, 1974, **34**, 1.
[472] Yu. A. Zhdanov, Yu. E. Alekseev, and G. E. Guterman, *Doklady Akad. Nauk S.S.S.R.*, 1973, **211**, 1345.

Compounds with an R—C—N Branch

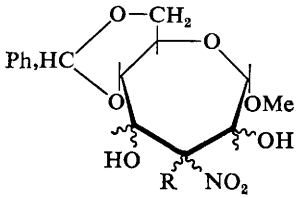
The sulphonylated cyanohydrin (268) has been converted into spiro-aziridine derivatives, which are valuable precursors of unsaturated sugars and branched-chain amino-sugars (see Scheme 91).[473]



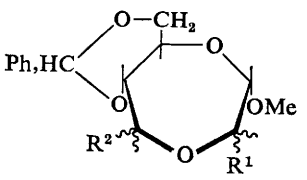
Reagents: i, $LiAlH_4$ (R = H); ii, RMgX (R = Me or Et); iii, H_2; iv, Ac_2O–py; v, HNO_2

Scheme 91

Periodate-oxidized methyl α-L-rhamnopyranoside has been condensed with nitroethane to furnish, *inter alia*, methyl 6-deoxy-3-*C*-methyl-3-*C*-nitro-α-L-glucopyranoside, which was converted into the 2-deoxy-derivative *via* the glycal route.[474] The base-catalysed condensation of nitroethane and higher nitroalkanes with periodate-oxidized methyl 4,6-*O*-benzylidene-α-D-glucopyranoside gave either branched-chain nitroseptanosides (269) or dioxepan derivatives [(270) and (271)], depending on the reagent and the conditions used.[475] Michael



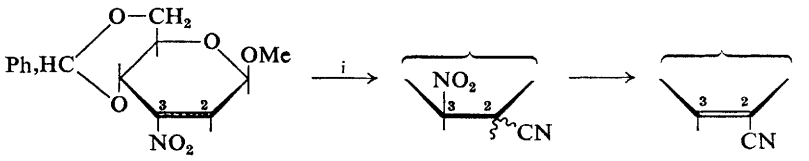
(269) R = Me or Et



(270) R^1 = $CHRNO_2$; R^2 = OH
(271) R^1 = OH; R^2 = $CHRNO_2$

addition of cyanide ion to sugar nitro-olefins yielded adducts that afforded cyano-olefins on loss of nitrous acid; thus, the 3-nitro-olefin (272) was transformed into the 2-cyano-sugar (Scheme 92).[476]

[473] J. M. Bourgeois, *Helv. Chim. Acta*, 1974, **57**, 2553.
[474] J. S. Brimacombe and L. W. Doner, *J.C.S. Perkin I*, 1974, 62.
[475] M. E. Butcher and J. B. Lee, *J.C.S. Chem. Comm.*, 1974, 1010.
[476] H. Paulsen and W. Greve, *Chem. Ber.*, 1974, **107**, 3013.



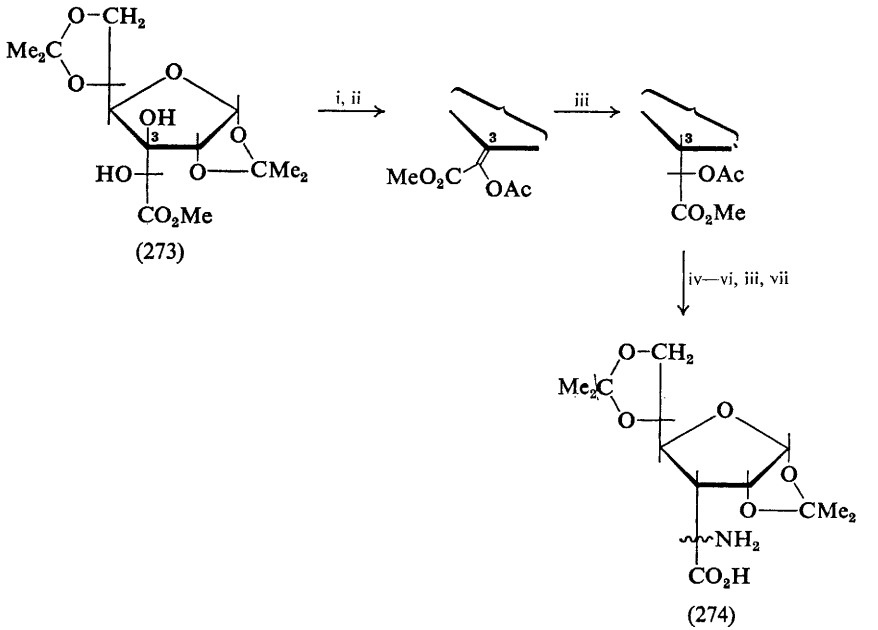
(272)

Reagents: i, HCN–Et_3N

Scheme 92

Compounds with an R^1—C—R^2 Branch

In pursuing their investigations of glycosyl α-amino-acids, Rosenthal's group has reported the synthesis of D- and L-2-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)glycine (274) from the branched-chain hydroxy-ester (273) by



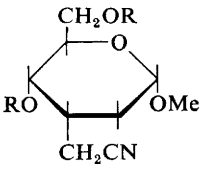
(273) (274)

Reagents: i, Ac_2O–py; ii, $SOCl_2$; iii, H_2–Pd; iv, NaOMe; v, MsCl or TsCl–py; vi, NaN_3–DMF; vii, NaOH

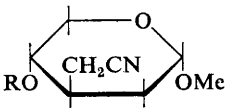
Scheme 93

way of an intermediate 3-*C*-methylene derivative (Scheme 93).[477] The *p*-chlorobenzoylated 3-*C*-(cyanomethyl)glycosides (275)[444] and (276)[445] were also prepared and converted into α- and β-nucleosides by fusion with 2,6-dichloropurine; the unsaturated sugar (277) was also isolated from the former reaction.

[477] A. Rosenthal and C. M. Richards, *Carbohydrate Res.*, 1973, **31**, 331.

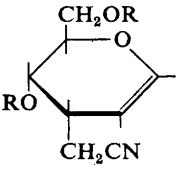


(275)



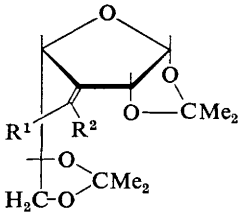
(276)

R = *p*-ClC_6H_4CO

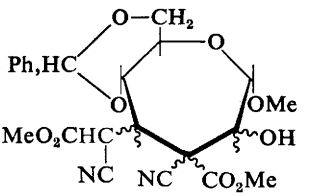


(277)

Tronchet's group has now published details of their studies on cyano- and methylthio-methylene derivatives obtained from 1,2-*O*-isopropylidene-α-D- and -L-*glycero*-tetros-3-ulose[478] and has also reported the synthesis of a series of 3-deoxy-3-*C*-halogenomethylene-α-D-*xylo*-hexofuranoses (278).[479]



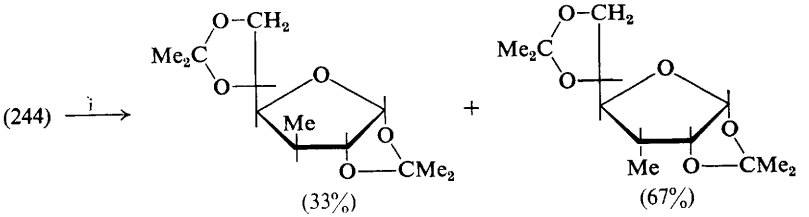
(278) R^1 = H; R^2 = Cl, Br, or I
R^1 = Cl, Br, or I; R^2 = H
R^1 = R^2 = Cl or Br



(279)

Cyanoacetate has been condensed with sugar dialdehydes (*e.g.* periodate-oxidized methyl 4,6-*O*-benzylidene-α-D-glucopyranoside) in a Knoevenagel reaction to give such branched-chain septanosides as (279).[480] A Knoevenagel reaction furnishing the acyclic *C*-methylene derivative (247) has been noted in Chapter 14.[455]

The dithiolylidene derivative (244) has been prepared by treatment of the corresponding glycosulose with tributylphosphine, carbon disulphide, and dimethyl acetylenedicarboxylate.[481] Desulphurization of (244) over Raney nickel gave a 2 : 1 mixture of isomeric 3-*C*-methyl derivatives (Scheme 94).



Reagent: i, Raney Ni

Scheme 94

The synthesis of deoxy-*C*-ethoxycarbonylmethyl sugars from oxiran derivatives is mentioned in Chapter 4.

[478] J. M. J. Tronchet and J. Tronchet, *Carbohydrate Res.*, 1974, **33**, 237.
[479] J. M. J. Tronchet and D. Schwarzenbach, *Carbohydrate Res.*, 1974, **38**, 320.
[480] M. E. Butcher and J. B. Lee, *Tetrahedron Letters*, 1974, 2663.
[481] J. M. J. Tronchet, T. Nguyen-Xuan, and M. Rouiller, *Carbohydrate Res.*, 1974, **36**, 404.

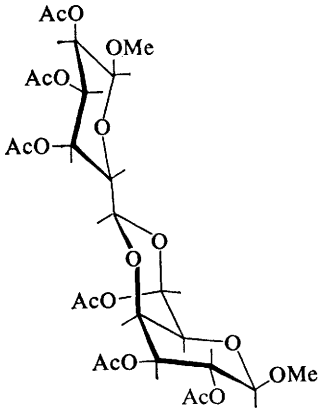
16

Aldehydo-sugars, Aldosuloses, Dialdoses, and Diuloses

The use of aldosuloses as intermediates in the synthesis of other sugars is covered in Chapters 7, 8, 10, and 15, and the preparation of derivatives of aldosuloses is also referred to in Chapter 22.

6-Aldehydo-sugars have been prepared by treating the corresponding amino-sugar derivative with ninhydrin.[482]

Methyl β-D-*galacto*-hexodialdo-1,5-pyranoside, obtained by oxidation of methyl β-D-galactopyranoside with D-galactose oxidase, has been characterized as the acetylated dimer (280).[483] In an investigation seeking to understand why

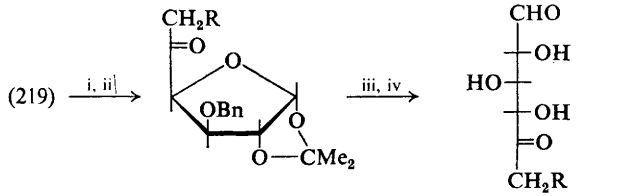


(280)

oxidation at C-6 of hexopyranosides labilizes the glycosidic linkage, a number of methyl hexodialdopyranosides have been prepared either by standard oxidative procedures or by cleavage of the corresponding methyl heptopyranoside with periodate ion.[484] The pentodialdose (219) has been used in a straightforward synthesis of the δ-dicarbonyl-sugar (281), as shown in Scheme 95.[485]

Partial oxidation and dimerization of lactose, to give di- and tetra-saccharides containing residues of D-*arabino*-hexosulose, have been shown to occur during ion-exchange chromatography employing alkaline solutions of borate.[486]

[482] A. R. Gibson, L. D. Melton, and K. N. Slessor, *Canad. J. Chem.*, 1974, **52**, 3905.
[483] A. Maradufu and A. S. Perlin, *Carbohydrate Res.*, 1974, **32**, 127.
[484] B. Pettersson and O. Theander, *Acta Chem. Scand.* (*B*), 1974, **28**, 29.
[485] D. C. Kiely, H. Walls, jun., and R. L. Black, *Carbohydrate Res.*, 1973, **31**, 387.
[486] B. N. White and R. Carubelli, *Carbohydrate Res.*, 1974, **33**, 366.

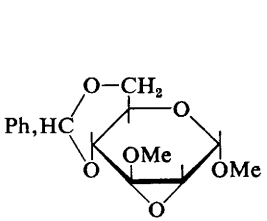


(281) R = H or Me

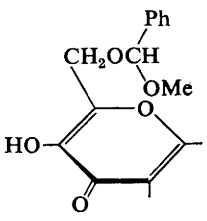
Reagents: i, RCH_2MgHal; ii, CrO_3–py; iii, H_2–Pd; iv, H_3O^+

Scheme 95

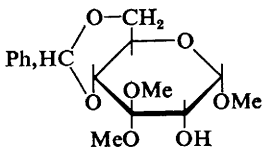
Treatment of methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside with butyl-lithium has provided a new route to methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (213).[487] The hexopyranosid-3-ulose (213) was also obtained by photolytic desulphonylation of methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-*ribo*-hexopyranosid-3-ulose (214) in the presence of triethylamine.[428] On heating with triethylamine in methanol, the α-keto-sulphonate (214) was transformed into (282)—(284), and similar products [(286) and (287)]



(282)



(283)



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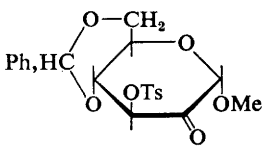
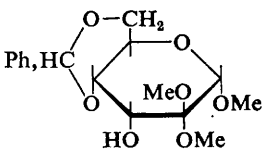


(285)



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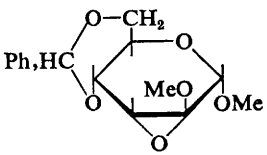
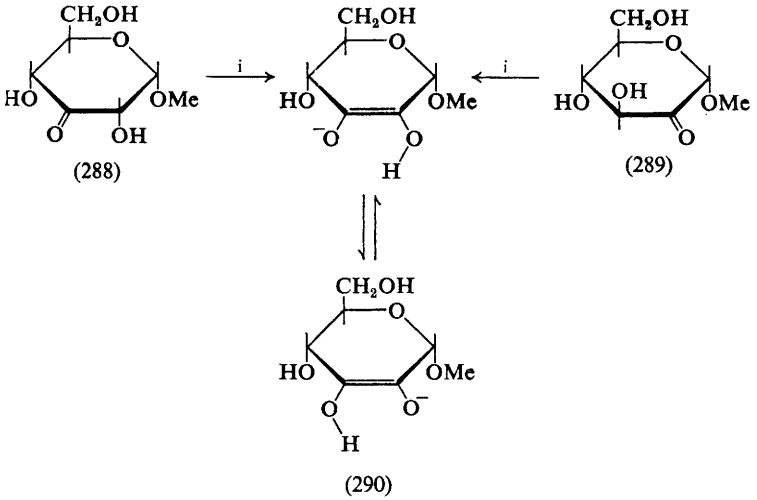


(287)

were obtained from an analogous reaction of the isomeric derivative (285).[488] The hexosiduloses (288) and (289) exhibited identical ^1H n.m.r. spectra in dilute alkaline solutions, indicating the formation of the common enolate ion (290) (Scheme 96), which decomposes in the presence of an excess of the base.[489]

[487] A. Klemer and G. Rodemeyer, *Chem. Ber.*, 1974, **107**, 2612.
[488] W. A. Szarek, A. Dmytraczenko, and J. K. N. Jones, *Carbohydrate Res.*, 1974, **35**, 203.
[489] J. Defaye, H. Driguez, and A. Gadelle, *Carbohydrate Res.*, 1974, **38**, C4.

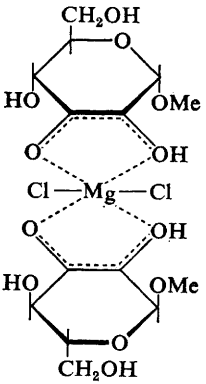


(288) (289) (290)

Reagents: i, NaOH–H_2O

Scheme 96

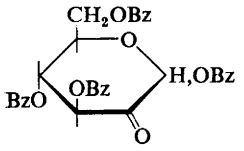
The addition of magnesium chloride stabilized the enolate by formation of a complex, suggested to have the structure (291).

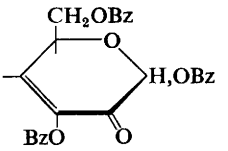


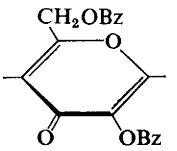
(291)

Both anomers of 1,3,4,6-tetra-*O*-benzoyl-D-*arabino*-hexopyranos-2-ulose (292) have been prepared by oxidation of the corresponding tetrabenzoate with ruthenium tetroxide.[490] The benzoylated hexopyranos-2-uloses readily eliminated benzoic acid on treatment with aqueous sodium hydrogen carbonate to give the hex-3-enosulose (293), whereas di-*O*-benzoylkojic acid (294) was obtained when they were heated in pyridine; the corresponding 1-acetates were less stable. Hydrolysis of the hexopyranosid-4-ulose (295) has provided a ready synthesis of maltol (296), a useful flavouring agent.[491] Treatment of methyl 2,4,6-tri-*O*-

[490] I. Lundt and C. Pedersen, *Carbohydrate Res.*, 1974, **35**, 187.
[491] R. K. Chawla and W. E. McGonigal, *J. Org. Chem.*, 1974, **39**, 3281.


(292)


(293)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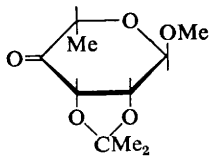
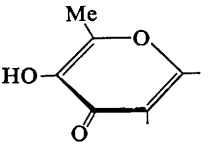

(295)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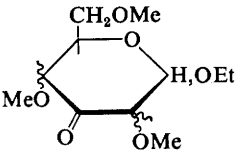
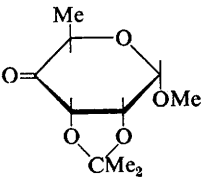
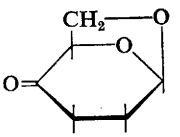

(297)

methyl-α-D-*ribo*-hexopyranosid-3-ulose with sodium ethoxide was earlier shown (see Vol. 6, p. 116) to yield derivatives of 1-deoxyhex-1-eno-3-uloses; other products (297) of the reaction have now been shown to result from addition of ethanol to the 1,2-unsaturated linkage.[492]

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*ribo*-hexopyranosid-4-ulose (298) has been prepared by oxidation of either methyl 6-deoxy-2,3-*O*-isopropylidene-α-


(298)

D-gulo- or -allo-pyranoside with ruthenium tetroxide.[493] Reduction of the 4-ulose (298) with sodium borohydride regenerated the aforementioned allo-pyranoside. Related reductions of a number of hexopyranosid-2-uloses are mentioned in Chapter 3.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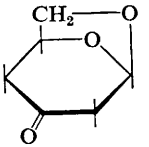
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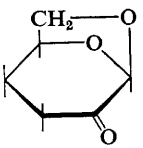
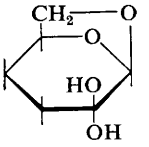
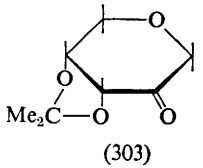

(301)


(302)

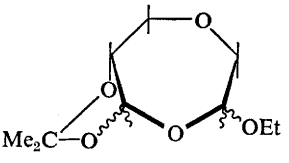
[492] L. Kenne, O. Larm, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 2797.
[493] P. M. Collins and B. R. Whitton, *Carbohydrate Res.*, 1974, **33**, 25.

The isomeric 1,6-anhydro-β-D-*glycero*-hexopyranosuloses (299)—(301) have been prepared, and the 2-ulose (301) was shown to exist in equilibrium with the hydrated form (302) in aqueous ethanol.[275] Reduction of each of these uloses with sodium borohydride yielded 84, 44, and 89%, respectively, of the equatorial alcohol.

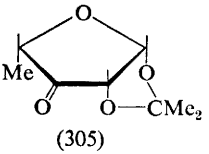
Photolysis of the 1,5-anhydropentulose (303) in ethanol–ether furnished a mixture of two dioxepans (304), whereas photolysis of the 3-ulose derivative (305) in methanol gave isomeric dioxans (306).[494] The photolytic addition of methanol to the hex-1-enopyranosid-3-ulose derivative (307) afforded the 2,6-anhydrohept-4-uloses (308) and (309) in the ratio of 7 : 3.[495]



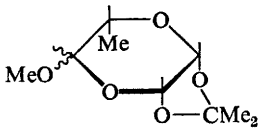
(303)



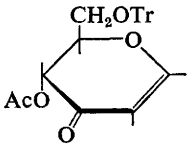
(304)



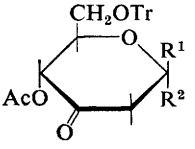
(305)



(306)



(307)



(308) R^1 = H; R^2 = CH_2OH
(309) R^1 = CH_2OH; R^2 = H

On heating in aqueous pyridine, methyl 6-deoxy-2,3-*O*-isopropylidene-β-L-*lyxo*-hexopyranosid-4-ulose was more extensively epimerized at C-5 than the corresponding α-glycoside (*cf.* Vol. 6, p. 115), as would be expected from conformational considerations.[319] Methyl 4-*O*-α-D-glucopyranosyl-β-D-*ribo*-hexopyranosid-3-ulose hexa-acetate (prepared from maltose) similarly gave a mixture of C-2 epimers on heating in pyridine.[185]

Some approaches to the synthesis of anil derivatives of methyl 4,6-*O*-benzylidene-α-D-*erythro*-hexopyranosid-2,3-diulose are reported in Chapter 9 (see Scheme 54), and the conversion of nitro-oxiran derivatives of sugars into glycosuloses is mentioned in Chapter 4. The degradation of hexodiulosonic acid derivatives to tetruloses and the synthesis of nucleoside derivatives of glycosuloses are referred to in Chapters 2 and 21, respectively.

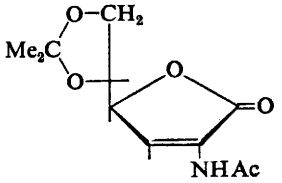
[494] P. M. Collins, N. N. Oparaeche, and B. R. Whitton, *J.C.S. Chem. Comm.*, 1974, 292.
[495] D. L. Walker, B. Fraser-Reid, and J. K. Saunders, *J.C.S. Chem. Comm.*, 1974, 319.

17
Sugar Acids and Lactones

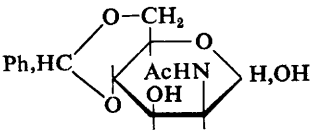
Aldonic Acids

In continuing his studies (Vol. 7, p. 128) on the hydrolysis of aldonolactones, Pocker has examined the hydrolyses of 2,3,4,6-tetra-*O*-methyl-D-glucono- and -mannono-1,5-lactones and a 2-deoxy-derivative thereof.[496] Whereas the hydrolysis of 2,3,4,6-tetra-*O*-methyl-D-mannono-1,5-lactone exhibited general base catalysis only, hydrolyses of the other lactones showed general base and general acid catalysis. The effects of the half-chair conformation and the substituents at C-2 on the hydrolytic behaviour of the lactones were discussed.

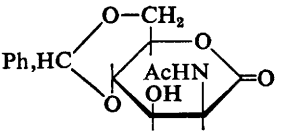
Several reports of unsaturated aldonolactones have appeared during the past year. Thus, D-glucono-1,5-lactone gave the unsaturated derivative (233) on treatment with a large excess of benzoyl chloride in pyridine,[450] and β-elimination to give the enono-1,4-lactone (310) occurred when 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone was treated with an excess of toluene-*p*-sulphonyl chloride in pyridine.[497] Although the oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose with bromine–cadmium carbonate gave the corresponding 1,5-lactone initially, it was transformed into 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-gluconic acid during processing. Silver carbonate on Celite (the Fetizon reagent) has been used to oxidize selectively such partially protected 2-acetamido-2-deoxypyranoses as (311) to the lactone (312),



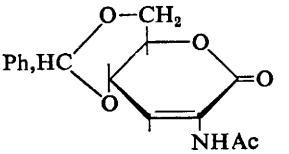
(310)



(311)



(312)



(313)

[496] Y. Pocker and E. Green, *J. Amer. Chem. Soc.*, 1974, **96**, 166.
[497] N. Pravdic, E. Zissis, M. Pokorny, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1974, **32**, 115.

but the unsaturated lactone (313) was also formed.[498] Partial *gluco*-to-*manno* epimerization was noted during the oxidation of such derivatives as 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose.

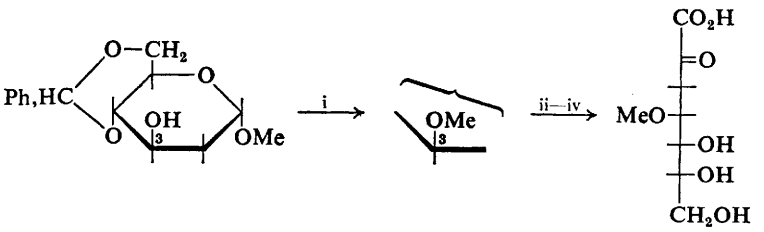
The chiroptical properties of 2-*C*-methylpentono-1,4-lactones are referred to in Chapter 25. The incorporation of deuterium and tritium into the saccharinic acids obtained from the degradations of maltose, turanose, and inulin in aqueous barium deuteroxide[499] and barium tritioxide,[500] respectively, has been examined. The position and extent of deuterium (tritium) incorporation into the isolated acids (3-deoxy-2-*C*-hydroxymethyl-D-*erythro*- and -*threo*-pentonic acids, 3-deoxy-D-*ribo*- and -*arabino*-hexonic acids, and 2-*C*-methyl-D-ribonic acid) were examined by mass-spectral and n.m.r. methods. A nucleoside possessing a carboxy-branch at C-2 of the sugar residue is described in Chapter 21.

Crystalline cellobiono-1,5-lactone has been prepared; its growth-inhibitory activity in standard *Avena* coleoptile section tests was comparable to the activities of D-galactono-1,5- and D-glucurono-6,3-lactones.[501] Syntheses of vinyl D-gluconate, from 3,4:5,6-di-*O*-isopropylidene-D-gluconic acid and acetylene [502] and of a lactam derived from 5-amino-5-deoxy-D-gluconic acid (Chapter 12) have been reported; the lactam is a powerful inhibitor of β-glucuronidase. The inhibitory activity of 2-acetamido-2-deoxy-D-gluconolactones and their isopropylidene derivatives against the 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymus has been examined.[503] Whereas the 1,5-lactone is highly active, the 1,4-lactone exhibited a weak activity that increased significantly with time, due mainly to equilibration with the 1,5-lactone.

The formation of complexes between aldonolactones and borate is referred to in Chapter 18, while the synthesis of 2-amino-2-deoxy-D(or L)-gluconic acid by way of the Ugi reaction is noted in Chapter 10.

Ulosonic Acids

3-Deoxy-4-*O*-methyl-D-*arabino*-2-heptulosonic acid (314) has been synthesized by the route shown in Scheme 97; contrary to prevailing opinion, (314) gave a



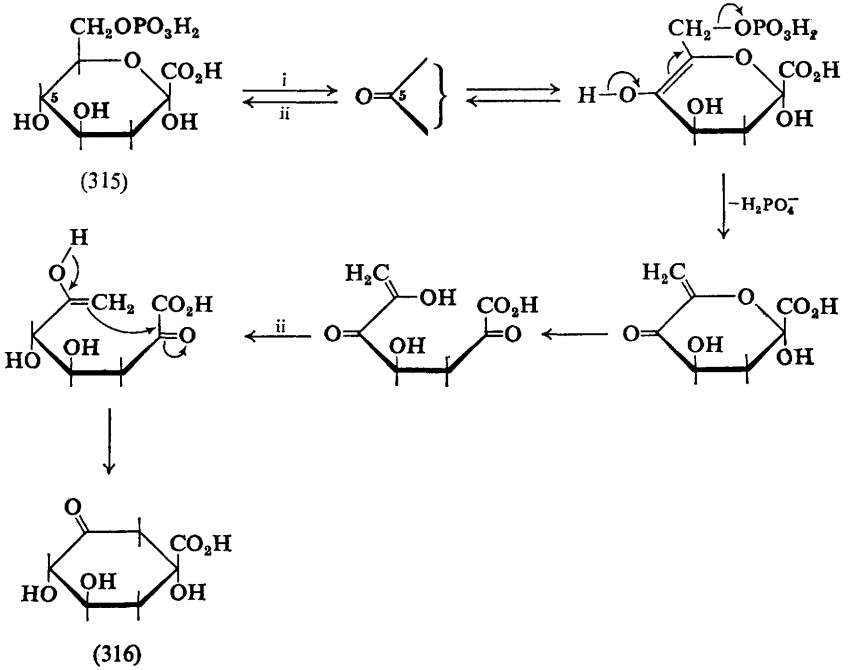
(314)

Reagents: i, MeI–Ag_2O; ii, H^+; iii, KCN; iv, V_2O_5

Scheme 97

[498] N. Pravdic, B. Danilov, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1974, **36**, 167.
[499] R. F. Burn and P. J. Somers, *Carbohydrate Res.*, 1973, **31**, 289.
[500] R. F. Burn and P. J. Somers, *Carbohydrate Res.*, 1973, **31**, 301.
[501] H. W. Diehl, M. Pokorny, E. Zissis, R. K. Ness, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1974, **38**, 364.
[502] V. L. Lapenko and A. I. Slivkin, *Monomery. Vysokomol. Soedinenii*, 1973, 103 (*Chem. Abs.*, 1974, **80**, 108 795d).
[503] M. Pokorny, E. Zissis, H. G. Fletcher, jun., and N. Pravdic, *Carbohydrate Res.*, 1974, **37**, 321.

positive reaction with periodate–thiobarbiturate (the Warren assay), indicating that this reaction is unreliable for the quantitative estimation of 3-deoxy-2-aldulosonic acids of undetermined substitution pattern.[504] The degradation of aldulosonic acids with alkaline hydrogen peroxide has been studied by Isbell and his colleagues (see Chapter 22).



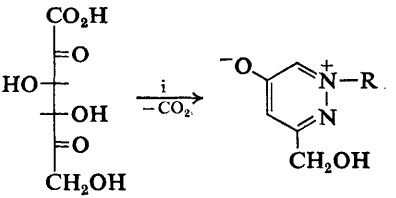
(315)

(316)

Reagents: i, NAD^+; ii, NADH

Scheme 98

The conversion of 3-deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (315) into 3-dehydroquinic acid (316) is an important pathway for the biosynthesis of aromatic amino-acids. The results of labelling studies are considered to be consistent with the reactions shown in Scheme 99 for this conversion.[505]



Reagent: i, $RNHNH_2$

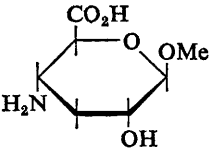
Scheme 99

[504] D. Charon and L. Szabo, *Carbohydrate Res.*, 1974, **34**, 271.
[505] P. Le Maréchal and R. Azerad, *Compt. rend.*, 1974, **278**, *C*, 1251.

D-*threo*-Hex-2,5-diulosonic acid reacted with monosubstituted hydrazines to give pyridazinium hydroxides, representatives of an unusual class of zwitterionic heterocyclic compound (Scheme 99).[506]

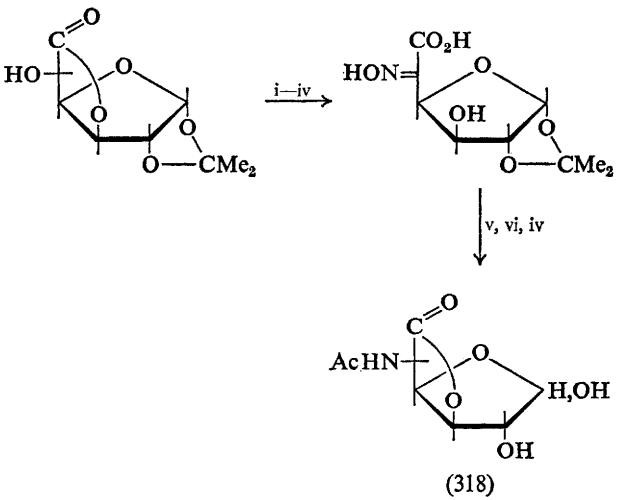
Uronic Acids

Methyl 4-amino-3,4-dideoxy-β-D-*ribo*-hexopyranoside and the uronic acid (317) thereof have been synthesized by way of a series of standard transformations on



(317)

1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose,[507] and a seven-step sequence has been used to prepare 5-acetamido-5-deoxy-D-glucurono-6,3-lactone (318) from an accessible precursor (Scheme 100).[508] Catalytic oxidation of (318) in the presence of sodium hydrogen carbonate furnished the



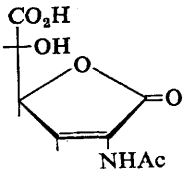
(318)

Reagents: i, CrO_3–EtOAc; ii, NaOH; iii, NH_2OH; iv, H^+; v, H_2–Pt; vi, Ac_2O

Scheme 100

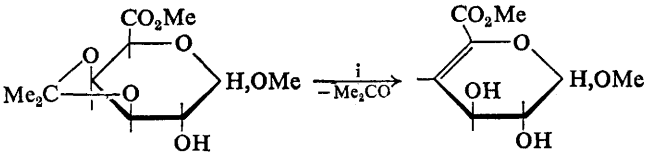
unsaturated saccharinic acid (319). 3-Amino-3,4-dideoxy-D-*xylo*-hexuronic acid has been identified as a constituent of ezomycins A_1 and A_2 (see Chapter 20), and reference is made in Chapter 15 to the synthesis of 3-*C*-hydroxymethyl-D-riburonic acid.

[506] K. Imada, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1732.
[507] T. M. K. Chiu, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1974, **32**, 211.
[508] K. Ochi and K. Okui, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2223.



(319)

β-Elimination reactions in alduronic acid derivatives have been reviewed,[454] and derivatives of methyl 3,4-*O*-isopropylidene-D-galacturonate have been shown to undergo base-catalysed β-elimination reactions with deacetalation (Scheme 101).[509]



Reagent: i, NaOMe–MeOH

Scheme 101

2-Amino-2-deoxyguluronic acid, which until now has been found in Nature only in *Vibrio parahaemolyticus*, has also been identified in cell-wall hydrolysates of *Halococcus* sp. strain 24, an extremely halophilic bacterium.[510] The occurrence of iduronic acid as a constituent of the 'type-specific' polysaccharide of *Clostridium perfringens* Hobbs 10 has been established.[511] In neither case was it possible to decide the configuration of the hexuronic acid moiety.

Isbell's investigations of the degradation of carbohydrates with alkaline hydrogen peroxide have been extended to alduronic acids (see Chapter 22), and the association between europium ions and sodium (methyl α-D-galactopyranosid)uronate has been studied by n.m.r. spectroscopy (see Chapter 18).

Ascorbic Acid

Molecular-orbital calculations have been presented for ascorbic acid, ascorbate, and several related compounds; calculated u.v.-absorption parameters were in good agreement with values determined experimentally.[512] The e.s.r. spectra of L-ascorbic acid radicals in aprotic solvents have been examined[513] (see also Chapter 23).

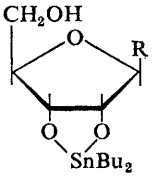
Improved procedures for the preparation of L-ascorbate 2-sulphate have been reported; the most efficient procedure involved heating L-ascorbate with trimethylamine–sulphur trioxide at pH 9.5—10.5.[514]

Reference to 2,3-diamino-2,3-dideoxy-L-ascorbic acid is made in Chapter 8.

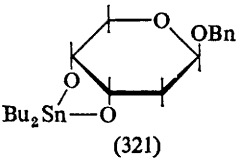
[509] P. Kováč, J. Hirsch, and V. Kováčik, *Carbohydrate Res.*, 1974, **32**, 360.
[510] R. Reistad, *Carbohydrate Res.*, 1974, **36**, 420.
[511] L. Lee and R. Cherniak, *Carbohydrate Res.*, 1974, **33**, 387.
[512] E. Flood and P. N. Skancke, *Acta Chem. Scand.*, 1973, **27**, 3069.
[513] Y. Kirino, *Chem. Letters*, 1974, 153 (*Chem. Abs.*, 1974, **80**, 96 271q).
[514] P. A. Seib, Y. T. Liang, C. H. Lee, R. C. Hoseney, and C. W. Deyoe, *J.C.S. Perkin I*, 1974, 1220.

18
Inorganic Derivatives

In continuing their investigations of the reactions of nucleosides with novel reagents, Moffatt and his co-workers have prepared a series of 2′,3′-*O*-(dibutylstannylene) derivatives (320) by treating the nucleoside in hot methanol with dibutyltin oxide; the products were soluble in hot methanol, and crystallized on removal of the solvent.[515] The organotin derivatives were generally fairly soluble in alcohols, but were hydrolysed on t.l.c. plates, and gave recognizable molecular ions on mass spectrometry; however, well-resolved ^{1}H n.m.r. spectra could not be



(320) R = uracil, cytosine, adenine, or hypoxanthine

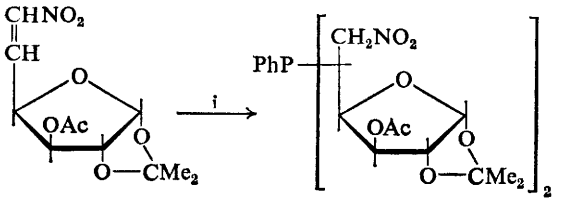


(321)

obtained. The dibutylstannylene group did not function as a protecting group in the reactions with either acyl chlorides or anhydrides, but activated O-2′ and O-3′ towards acylation. A related study has established that the *cis*-hydroxy-groups of benzyl 2-deoxy-β-D-*erythro*-pentopyranoside react with dibutyltin oxide to give the 3,4-*O*-(dibutylstannylene) derivative (321).[516] A series of 4- and 6-substituted D-glucopyranosides likewise reacted to furnish products containing equimolar proportions of the sugar moiety and tin, but structures were not assigned to these organotin derivatives and the possibility exists that they are polymeric.[516a]

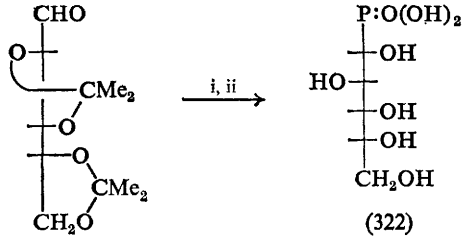
The first synthesis of 'hexopyranose' derivatives containing a phosphorus atom in the ring is noted in Chapter 12 (see Scheme 72); a number of phosphorus-containing derivatives of D-glucofuranose (Scheme 102) were also obtained in the course of this work.[423] Studies of sugars containing a carbon–phosphorus bond by Paulsen's group have led to the preparation of the (1*S*)-phosphonic acid (322) (Scheme 103), and a derivative thereof has been converted into dimethyl (4*S*)-(1,2-*O*-isopropylidene-β-D-threofuranosyl)phosphonate (323) (Scheme 104).[517]

[515] D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 24.
[516] S. David, *Compt. rend.*, 1974, **278**, *C*, 1051.
[516a] S. David and A. Thieffry, *Compt. rend.*, 1974, **279**, *C*, 1045.
[517] H. Paulsen and H. Kuhne, *Chem. Ber.*, 1974, **107**, 2635.



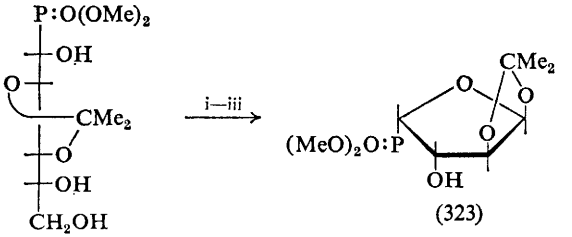
Reagent: i, $PhPH_2$

Scheme 102



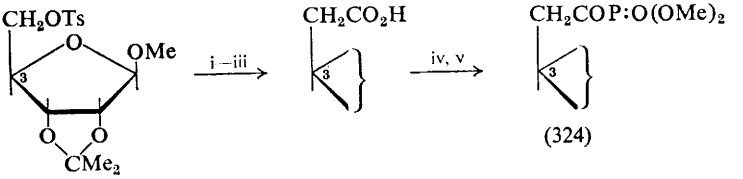
Reagents: i, $HP{:}O(OR)_2$–NaOMe; ii, H^+

Scheme 103



Reagents: i, IO_4^-; ii, H^+; iii, Me_2CO–$CuSO_4$

Scheme 104



Reagents: i, NaI–DMF; ii, NaCN–DMF; iii, KOH; iv, $SOCl_2$; v, $(MeO)_3P$

Scheme 105

The synthesis of an isosteric phosphonate analogue (324) of D-ribose 5-phosphate bearing a carbonyl group α to the phosphorus atom has been described (Scheme 105).[262]

An attempt to use glycosyl phosphonium salts in the synthesis of glycosides is noted in Chapter 3, and a report on organomercury derivatives of sugars is contained in Chapter 7.

Oxygen-bonded Compounds

Angyal has summarized his findings on the nature of the complexes formed in aqueous solution between polyhydroxy-compounds and metal ions, particularly those of the alkaline-earth metals.[518] The following points are worthy of note: sugars and cyclitols with an *ax.,eq.,ax.* arrangement of hydroxy-groups on a six-membered ring or a *cis,cis* arrangement on a five-membered ring readily form complexes in aqueous solution; complex formation alters the anomeric equilibrium of such sugars as D-allose and D-gulose, and the conformational equilibrium of such compounds as methyl β-D-ribopyranoside and β-D-lyxopyranose; the equilibrium composition of methyl glycosides in methanol is affected by the presence of a salt (*e.g.* $CaCl_2$). A study of the complexes formed between alditols and cations has also been undertaken,[519] and these and other results have been comprehensively reviewed by Angyal.[520] A *threo,threo* configuration at three consecutive carbon atoms of the alditol is favourable for complex formation, an *erythro,threo* configuration is somewhat less favourable, and *erythro,erythro* configurations do not give rise to stable metal complexes. Similar considerations apply when the terminal hydroxy-group is involved in complex formation. Angyal's group has also examined the stereochemistry of complex formation of polyhydroxy-compounds with borate and periodate anions.[521] In alkaline solution, periodate ions formed complexes with three consecutive hydroxy-groups in an *ax.,eq.,ax.* arrangement but not with three *syn*-axial hydroxy-groups, whereas the reverse situation was encountered with borate anions. Thus, *cis*-inositol gave a 1,2,3-periodate and a 1,3,5-borate complex. Cations (*e.g.* Ca^{2+}, Mg^{2+}) formed complexes with either type of conformation, but complex formation provided little energy towards achieving it by ring inversion. A useful review of complex formation of polyhydroxy-compounds with borate anions has clarified certain aspects of the interactions involved.[522]

Complex formation with borate anions has been used in conjunction with c.d. measurements to determine the configurations at C-2 of a series of aldono-1,4-lactones,[523] and n.m.r. methods have been used to study the association of europium ions with sodium (methyl α-D-galactopyranosid)uronate.[524] In the latter study, it was concluded that the metal cation complexes with the carboxy-group, the ring-oxygen atom, and O-4.

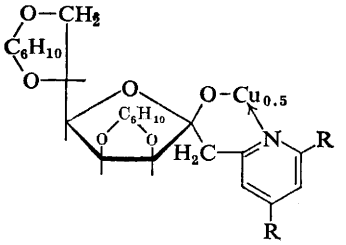
[518] S. J. Angyal, in 'Carbohydrates in Solution', Advances in Chemistry Series, No. 117, The American Chemical Society, 1973, p. 106.
[519] S. J. Angyal, D. Greeves, and J. A. Mills, *Austral. J. Chem.*, 1974, **27**, 1447.
[520] S. J. Angyal, *Tetrahedron*, 1974, **30**, 1695.
[521] S. J. Angyal, D. Greeves, and V. A. Pickles, *Carbohydrate Res.*, 1974, **35**, 165.
[522] T. E. Acree, in 'Carbohydrates in Solution', Advances in Chemistry Series, No. 117, The American Chemical Society, 1973, p. 208.
[523] H. Meguro, A. Tagiri, and K. Tuzimara, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 595.
[524] T. Anthonsen, B. Larsen, and O. Smidsrod, *Acta Chem. Scand.*, 1973, **27**, 2671.

Metal complexes of glycoside derivatives have been selectively methylated and acetylated; for example, various proportions of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, sodium hydride, and copper(II) chloride in either THF or DMF gave different chelated forms, which preferentially yielded 2- or 3-mono- or 2,3-di-*O*-methyl ethers when treated with methyl iodide.[525] 2,3,4(6)-Tri- and 2,3,4,6-tetra-*O*-methyl ethers were similarly prepared by methylation of specific copper complexes of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside.

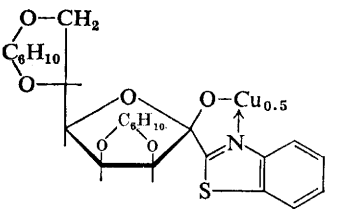
The hydrolysis of 8-quinolyl β-D-glucopyranoside in the presence of copper(II) ions is referred to in Chapter 3, and a complex formed by magnesium ions and hexopyranosiduloses is noted in Chapter 16.

Complexes with Nucleosides and Related Compounds

The complexes (325) and (326) were readily formed by treatment of the appropriate nitrogen heterocycle with copper(II) acetate in methanol, and their struc-



(325) R = H or Me



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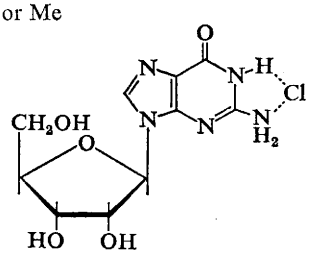


(327)

tures were revealed by physical methods (i.r. and c.d. absorptions, magnetic and dipole moments).[526] Charge-transfer complexes formed between nucleosides and the 2,2′-bipyridylcopper(II) complex, and the binding of methylmercury(II) ions to nucleosides, are referred to in Chapter 21.

The selective attachment of a heavy metal to one of the four heterocyclic bases commonly found in DNA is of particular interest for studies of the polymeric structure by electron microscopy. With this in mind, the interactions of a series of ^{14}C-labelled deoxyribonucleosides with *cis*-β-[cobalt(triethylenetetramine)-Cl_2]Cl have been examined.[527] Deoxyadenosine did not interact with the poly-

[525] E. Avela, *Sucr. Belge Sugar Ind. Abs.*, 1973, **92**, 337.

[526] Yu. A. Zhdanov, O. A. Osipov, V. P. Grigoriev, A. D. Garnovsky, Yu. E. Alexeev, V. G. Alexeeva, N. M. Gontmacher, P. A. Perov, V. G. Zaliotov, V. N. Fomina, T. A. Useman, O. N. Nechaeva, and V. N. Mirny, *Carbohydrate Res.*, 1974, **38**, C1.

[527] L. G. Marzilli, T. J. Kistenmacher, P. E. Darcy, D. J. Szalda, and M. Beer, *J. Amer. Chem. Soc.*, 1974, **96**, 4686.

amine, whereas thymidine, deoxycytidine, and deoxyguanine reacted to an extent related to both the co-ordinating affinity of the metal ion for the base and the ability of the base to accept interligand hydrogen bonds. A related study using alkaline-earth metal chlorides has indicated that the charge-reversed chelate (327) is formed between guanosine and chloride ion, but no bonding with the metal ion was detected.[528]

[528] C.-H. Chang and L. G. Marzilli, *J. Amer. Chem. Soc.*, 1974, **96**, 3656.

19
Cyclitols

A review (in Russian) has appeared on the chemistry of *myo*-inositol.[529] Di- and tri-*O*-(indole-3-acetyl)-*myo*-inositols have been isolated from kernels of *Zea mays*,[530] and 6-*O*-β-D-galactopyranosyl-*myo*-inositol has been found in the mammary glands of rats;[531] this is the first reported occurrence of this glycoside in animals.

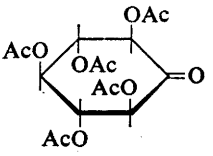
The rates and extents of complex formation of a series of *C*-methyl-, deoxy-*C*-methyl-, *C*-hydroxymethyl-, and *C*-methoxymethyl-inositols with borate anions have been found to be in accordance with values expected from conformational considerations.[532] The equilibrium constants for the formation of triaxial borate complexes were used to calculate the following values for the interaction energies, in aqueous solution, of a methyl group linked to a cyclitol: $C_{ax.}/H_{ax.}$ 3.8, C-1/O-2 1.9, and $C_{ax.}/O_{ax.}$ 6.8 kJ mol^{-1}; the last value is unexpectedly small when comparison is made with values (*ca.* 10 kJ mol^{-1}) derived for pyranose rings.

(±)-*myo*-Inositol 1-phosphate has been synthesized from (±)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol,[257] and a number of 5-nitro-2-furoylated inositols have been prepared and tested for antimicrobial activity, the *muco*-isomer being the most effective.[533] The *epi*-isomer was found to be the most effective antimicrobial agent of a series of *p*-hydroxybenzoylated inositols, which were obtained by esterification of inositols derived by appropriate reduction of inosose derivatives.[534]

The crystal and molecular structures of *myo*-inositol 2-phosphate have been determined, and they show that the phosphate group is attached to the axial oxygen atom.[535]

The reactions of *myo*-2-inosose penta-acetate (328) with diazoalkanes have been investigated; whereas the reaction with diazomethane[536] gave only the spiro-oxirans (329; R = H), that of higher diazoalkanes[536, 537] afforded mixtures of spiro-oxirans (329; R = Me, Et, *etc.*) and ring-expanded products (330) having an all-*trans* configuration; the ring-expanded products gave hemiacetals (331) on deacetylation.

[529] V. I. Shvets, *Uspekhi Khim.*, 1974, **43**, 1074 (*Chem. Abs.*, 1974, **81**, 136 390g).
[530] A. Ehmann and R. S. Bandurski, *Carbohydrate Res.*, 1974, **36**, 1.
[531] W. F. Naccarato and W. W. Wells, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1026.
[532] S. J. Angyal, J. E. Klavins, and J. A. Mills, *Austral. J. Chem.*, 1974, **27**, 1075.
[533] J. H. Sohn, Y. I. Kim, and V. R. Park, *Han'guk Sikp'um Kwahakhoe Chi*, 1973, **5**, 249 (*Chem. Abs.*, 1974, **80**, 108 787c).
[534] J. H. Sohn, *Han'guk Sikp'um Kwahakhoe Chi*, 1973, **5**, 240 (*Chem. Abs.*, 1974, **80**, 108 786b).
[535] C. S. Yoo, G. Blank, J. Pletcher, and M. Sax, *Acta Cryst.*, 1974, **B30**, 1983.
[536] A. Giddey, F. G. Cocu, B. Pochelon, and Th. Posternak, *Helv. Chim. Acta*, 1974, **57**, 1963.
[537] F. G. Cocu, B. Pochelon, A. Giddey, and Th. Posternak, *Helv. Chim. Acta*, 1974, **57**, 1974.



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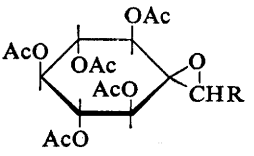

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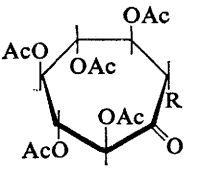
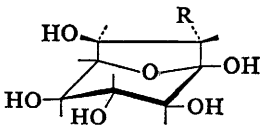


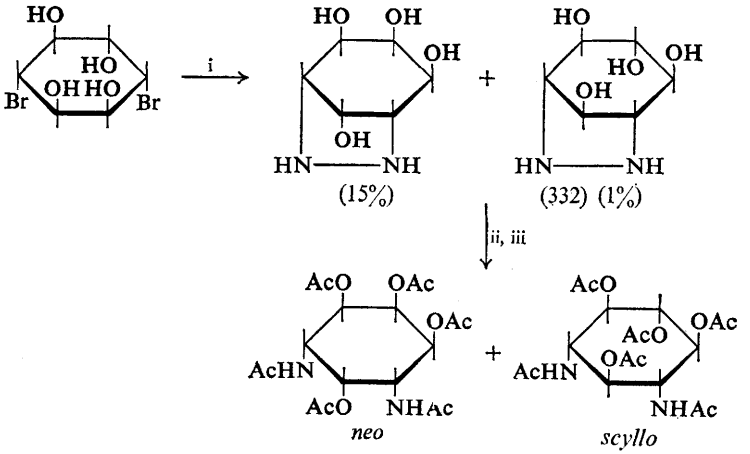
(330) R = Me, Et, *etc.*



(331) R = Me, Et, *etc.*

Amino-cyclitols

Diamino-inositols (inosadiamines) have been synthesized from hydrazino derivatives of inositols, as shown in Scheme 106, and a derivative of 4,6-diamino-

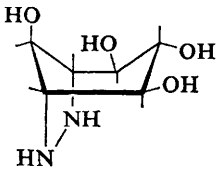


Reagents: i, NH_2NH_2; ii, H_2–Pt; iii, Ac_2O–py

Scheme 106

inositol was also prepared.[538] ^{1}H N.m.r. spectroscopic data for the hydrazino derivatives indicated that the *scyllo*-isomer (332) adopts a flattened-boat conformation (333) in preference to the all-axial chair form. Other diamino-inositols have been synthesized by way of the azidolysis of inositol disulphonates, providing the first reported syntheses of derivatives of *allo*-1,4-, *muco*-1,2-, and *chiro*-2,4-diamino-inositols; extensive use was made of the participation by neighbouring acetoxy-groups in these displacements.[539] The reaction of azide ions in 90%

[538] T. Suami, S. Ogawa, H. Uchino, and M. Uchida, *Bull. Chem. Soc. Japan*, 1973, **46**, 3840.
[539] T. Suami, S. Ogawa, S. Oki, and H. Sato, *Bull. Chem. Soc. Japan*, 1974, **47**, 1731.



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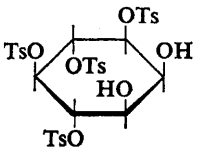

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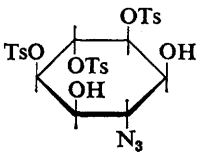
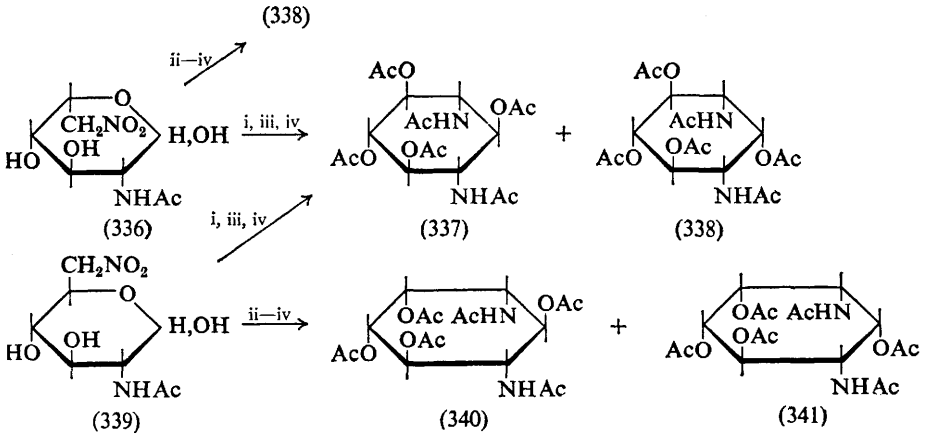


(335)

2-methoxyethanol with 1,4,5-, 1,4,6-, 1,5,6-, and 4,5,6-tri- and 1,4,5,6-tetra-sulphonates of *myo*-inositol has been used to prepare a number of mono-, di-, and tri-azido-inositol derivatives; acetylated derivatives of these sulphonates furnished products different from those obtained with the unacetylated compounds as a result of neighbouring-group participation.[539a] The 1,4,5,6-tetra-sulphonated derivative (334) reacted to give only the mono-azide (335), presumably by opening of an intermediate 3,4-oxiran.

Diamino-inositols have also been synthesized by way of base-catalysed cyclization of 2-acetamido-2,6-dideoxy-6-nitrohexoses.[540] Depending on the basic conditions used for cyclization, the L-*ido*-compound (336) furnished *scyllo*-1,3-diamino(streptamine) (337) and *myo*-1,3-diamino (338) derivatives, whereas the D-*gluco*-compound (339) yielded either (337) and (338) or 1L-*myo*-1,5-diamino (340) and 1L-*epi*-1,3-diamino (341) derivatives, respectively (Scheme 107).

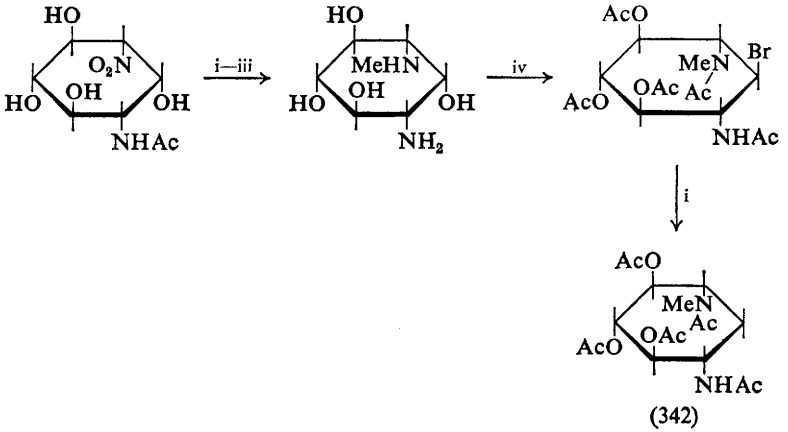


Reagents: i, $Ba(OH)_2$–H_2O; ii, MeONa–MeOH; iii, H_2–Pt; iv, Ac_2O–py

Scheme 107

Syntheses of neosamines B and C were also reported. The nitro-inositol yielding (338) was also converted into (−)-hyosamine penta-acetate (342) by a reaction sequence (Scheme 108) involving the selective displacement of an axial hydroxy-group with acetyl bromide–acetic anhydride.[541] An analogous approach was

[539a] T. Suami, S. Ogawa, S. Oki, and H. Kunitomo, *Bull. Chem. Soc. Japan*, 1974, **47**, 1737.
[540] S. Ogawa, K. L. Rinehart, jun., G. Kimura, and R. P. Johnson, *J. Org. Chem.*, 1974, **39**, 812.
[541] T. Suami, S. Ogawa, N. Tanno, M. Suguro, and K. L. Rinehart, jun., *J. Amer. Chem. Soc.*, 1973, **95**, 8734.

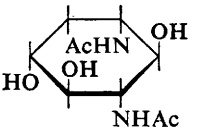


(342)

Reagents: i, H_2–Ni; ii, HCHO–H_2; iii, 6N-HCl; iv, AcBr–Ac_2O

Scheme 108

used to obtain (−)-4-deoxystreptamine (343) from 1,5-diamino-1,5-dideoxy-*myo*-inositol hexa-acetate (340); (343) proved to be enantiomeric with the compound obtained by degradation of streptomycin.



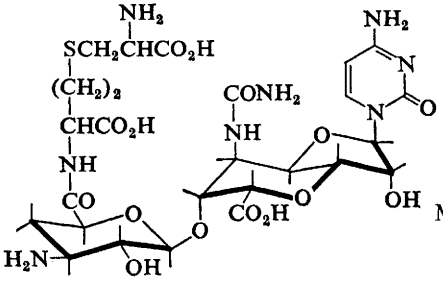
(343)

The electrophoretic behaviour of amino-inositols (inosamines) in borate buffer has been shown to depend very markedly on configuration, and effective separations of mixtures of amino-inositols can be achieved.[542] The probable sites of reaction between an amino-inositol and borate ions were identified for some of the isomers. Paper electrophoresis in non-complexing buffers provided evidence of the relative basicities of the amino-inositols, which exhibited enhanced basicity when a *syn*-diaxial effect operated in the preferred conformation. In other cases, an amino-group was found to be less basic in an axial than in an equatorial orientation, and the presence of two adjacent *cis*-hydroxy-groups ('*cis* effect') did not appear to be base-strengthening.

[542] J. L. Frahn and J. A. Mills, *Austral. J. Chem.*, 1974, **27**, 853.

20
Antibiotics

Ezomycin A_1 (344) has been identified as a nucleoside antibiotic containing, in addition to cytosine, 3,7-anhydro-5-deoxy-5-ureido-D-*threo*-D-*allo*-octofuranosyluronic acid and L-cystathionine, a novel aminohexuronic acid (ezoaminouronic acid) shown to be 3-amino-3,4-dideoxy-D-*xylo*-hexuronic acid.[543, 544] Aspiculamycin (345) has also been identified as a cytosine derivative containing 4-amino-4-



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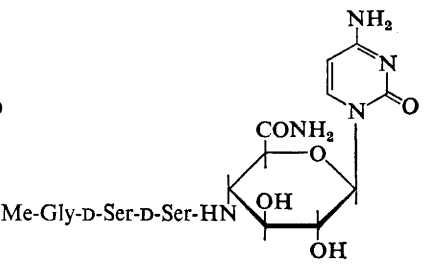

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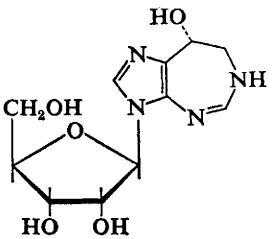


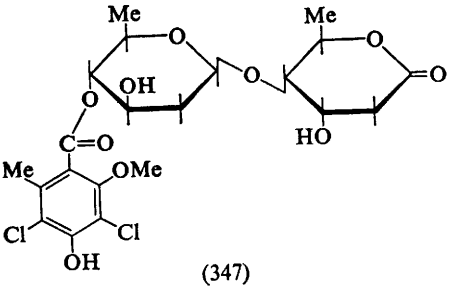
(346)

deoxy-β-D-glucopyranuronosylamide linked to a tripeptide through the 4-amino-group.[545] Coformycin (346), which is found with formycin in *Streptomyces kaniharaensis*, has been shown by *X*-ray analysis to contain an unusual 1,3-diazepine base;[546] Umezawa's group has synthesized this nucleoside antibiotic by an elegant ring-expansion on 9-β-D-ribofuranosylpurine.[547]

[543] K. Sakata, A. Sakurai, and S. Tamura, *Tetrahedron Letters*, 1974, 1533.
[544] K. Sakata, A. Sakurai, and S. Tamura, *Tetrahedron Letters*, 1974, 4327.
[545] T. Haneishi, A. Terahara, and M. Arai, *J. Antibiotics*, 1974, **27**, 334.
[546] H. Nakamura, G. Koyama, Y. Iitaka, M. Ohno, N. Yagisawa, S. Kondo, K. Maeda, and H. Umezawa, *J. Amer. Chem. Soc.*, 1974, **96**, 4327.
[547] M. Ohno, N. Yagisawa, S. Shibahara, S. Kondo, K. Maeda, and H. Umezawa, *J. Amer. Chem. Soc.*, 1974, **96**, 4326.

Two new antibiotics, hybrimycins C_1 and C_2, have been identified as analogues of paromycins I and II, respectively, in which a streptaminyl residue replaces one of 2-deoxystreptamine; selective hydrolysis of either with acid yielded hybrimycin C3, an analogue of paromamine.[548]

Flambamycin, a new antibiotic isolated from *Streptomyces hygroscopicus*, has been shown to belong to a family of structurally related carbohydrate antibiotics which includes curamycin, avilamycin, and everninomycins B and D.[549, 549a] Hydrolysis of flambamycin with acid liberated, *inter alia*, flambalactone (347) and flambatetraose isobutyrate (348), which contains D-evalose (6-deoxy-3-*C*-methyl-D-mannose), also found in everninomycin B. New antibiotics (349)



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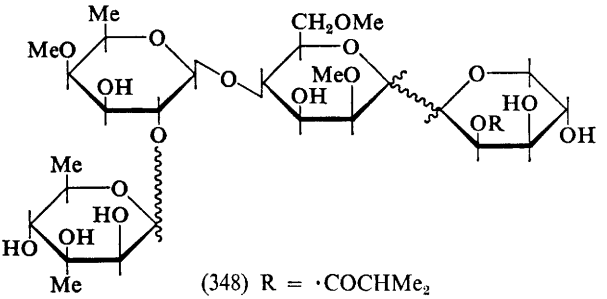
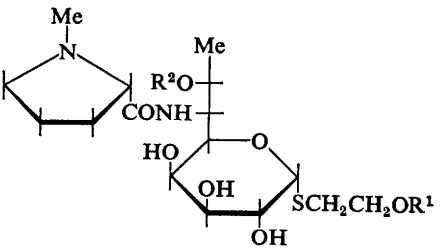
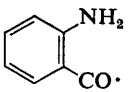


(348) R = ·COCHMe$_2$



(349) R^1 = ·COC_3H_7, ·COC_4H_9, or 
R^2 = H or Me

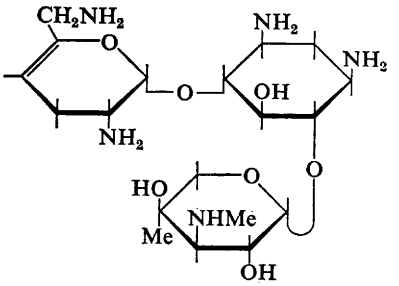
[548] W. T. Shier, P. C. Schaefer, D. Gottlieb, and K. L. Rinehart, jun., *Biochemistry*, 1974, **13**, 5073.

[549] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1974, 881.

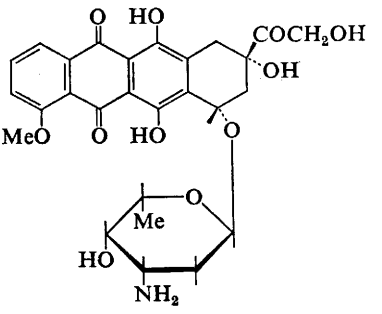
[549a] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1974, 882.

containing residues of either lincosamine or celestosamine (see below) have been isolated from cultures of *S. caelestis*.[550]

A detailed paper on the chemistry of sisomycin (350) has reported the complete elucidation of its structure [551] (see also Vol. 5, p. 131), and the antibiotics Bu-1975 C_1 and C_2 have been identified as 4′-deoxy-butirosins containing β-D-xylo- and -ribo-furanosyl residues, respectively.[552]

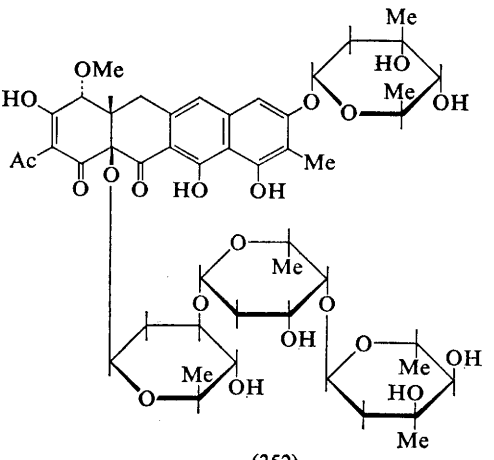


(350)



(351)

Adriamycin (doxorubicin) (351) has been shown to be an anthracycline antibiotic containing daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) as the sugar component,[553] and carminomycin, a new antibiotic isolated from *Actinomadura carminata*, was reported to be a related anthracycline antibiotic also containing daunosamine.[554] Chromocyclomycin (352) (from *Streptomyces*



(352)

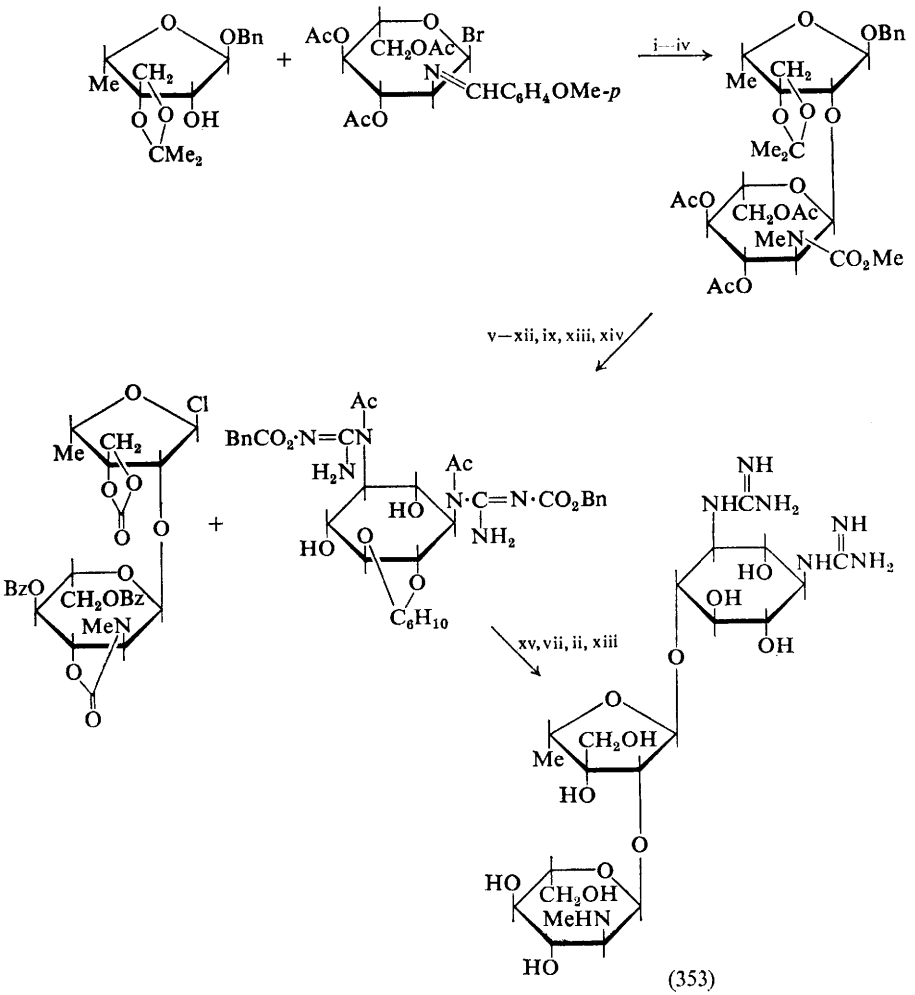
[550] A. D. Argoudelis and T. F. Brodasky, *J. Antibiotics*, 1974, **27**, 642.

[551] H. Reimann, D. J. Cooper, A. K. Mallams, R. S. Jarat, A. Yehaskel, M. Kugelman, H. F. Vernay, and D. Schumacher, *J. Org. Chem.*, 1974, **39**, 1451.

[552] M. Konishi, K. Numata, K. Shimoda, H. Tsukiura, and H. Kawaguchi, *J. Antibiotics*, 1974, **27**, 471.

[553] F. Arcamone, G. Cassinelli, G. Franceschi, S. Penco, C. Pol, S. Redaelli, and A. Selva, *Int. Symp. Adriamycin* (*Proc.*), 1971, 9 (*Chem. Abs.*, 1974, **80**, 83 521w).

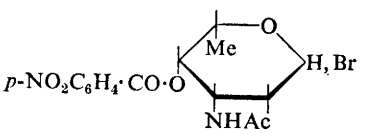
[554] M. G. Brazhnikova, V. B. Zbarskii, V. L. Ponomarenko, and N. P. Potapova, *J. Antibiotics*, 1974, **27**, 254.



(353)

Reagents: i, $Hg(CN)_2$–PhH; ii, 50% AcOH; iii, MeOCOCl; iv, MeI–Ag_2O–DMF; v, NaOMe–MeOH; vi, 0·5M-HCl; vii, $Ba(OH)_2$; viii, $Me_2C(OMe)_2$; ix, *p*-$NO_2C_6H_4OCOCl$; x, 25% AcOH–MeOH; xi, BzCl-py; xii, 75% AcOH; xiii, H_2–Pd–C; xiv, $SOCl_2$; xv, Ag_2CO_3–$AgClO_4$

Scheme 109



(354)

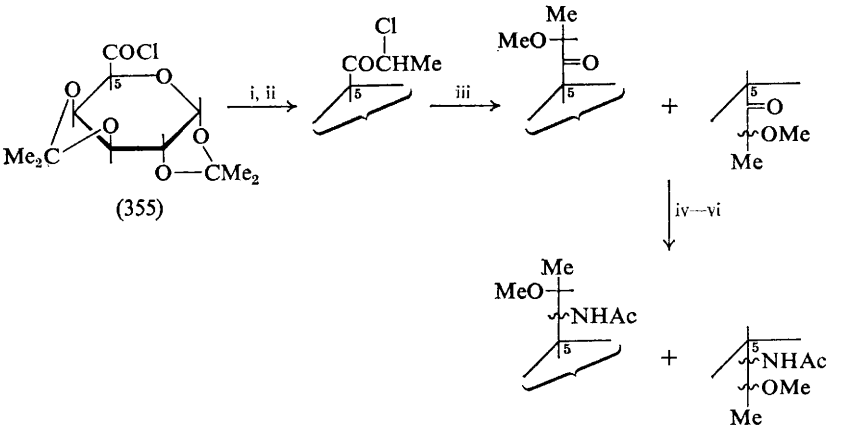
LA 7017) has been shown to contain two D-mycarosyl residues and two 2,6-dideoxyhexose residues attached to the tetracycline moiety.[555]

The complete structure of primycin, a macrolide antibiotic elaborated by *S. primycini*, has been elucidated by a combination of chemical and spectroscopic evidence; the presence of a D-arabinofuranosyl residue was demonstrated.[556, 557]

Structures have been proposed for the individual components of the antibiotic complex YL-704 obtained from *S. platensis*; all the components, which include platenomycin, contain a disaccharide comprising 4-*O*-acyl-L-mycarosyl-β-L-desosamine linked to the macrolide ring.[558, 559]

Methanolysis of the antibiotic YA-56 has indicated the presence of the disaccharide 6-deoxy-2-*O*-(3-*O*-carbamoyl-D-mannosyl)-L-gulose,[560] and further investigations of the structure of the aglycone portion of vancomycin have been reported.[561]

One of the highlights of the past year has been the synthesis of dihydrostreptomycin (353) by Umezawa and his colleagues; this first synthesis of an antibiotic of the streptomycin group provides an elegant illustration of the use and selective removal of protecting groups (see Scheme 109).[562] Daunomycin (daunorubicin) has been synthesized from daunomycinone and the daunosaminyl



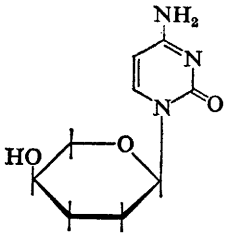
Reagents: i, $MeCHN_2$–Et_2O; ii, HCl–Et_2O; iii, BF_3–MeOH; iv, NH_2OH–py; v, $LiAlH_4$–THF; vi, Ac_2O–py

Scheme 110

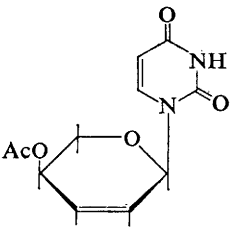
[555] Yu. A. Berlin, M. N. Kolosov, and I. V. Yartseva, *Khim. prirod. Soedinenii*, 1973, **9**, 539 (*Chem. Abs.*, 1974, **80**, 27 439p).
[556] J. Aberhart, R. C. Jain, T. Fehr, P. de Mayo, and I. Szilagyi, *J.C.S. Perkin I*, 1974, 816.
[557] T. Fehr, R. C. Jain, P. de Mayo, O. Motl, I. Szilagyi, L. Baczynskyj, D. E. F. Gracey, H. L. Holland, and O. B. MacLean, *J.C.S. Perkin I*, 1974, 836.
[558] A. Kinumaki, I. Takamori, Y. Sugawara, M. Suzuki, and T. Okuda, *J. Antibiotics*, 1974, **27**, 107.
[559] A. Kinumaki, I. Takamori, Y. Sugawara, Y. Seki, M. Suzuki, and T. Okuda, *J. Antibiotics*, 1974, **27**, 117.
[560] Y. Ohashi, S. Kawabe, T. Kono, and Y. Ito, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2379.
[561] K. A. Smith, D. H. Williams, and G. A. Smith, *J.C.S. Perkin I*, 1974, 2369.
[562] S. Umezawa, T. Tsuchiya, T. Yamasaki, H. Sano, and Y. Takahashi, *J. Amer. Chem. Soc.*, 1974, **96**, 920.

bromide (354) by a modified Koenigs–Knorr reaction that gave only the α-glycoside.[563]

Derivatives of celestosamine (6-amino-6,8-dideoxy-7-*O*-methyl-D-*erythro*-D-*galacto*-octose) have been synthesized from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranuronosyl chloride (355) (Scheme 110), and the amino-sugar was also obtained from lincosamine.[564] The nucleoside antibiotic pentopyranine A (356)



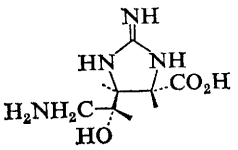
(356)



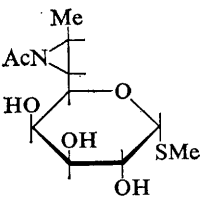
(357)

has been synthesized from N^4-anisoyl-1-(2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl)cytosine by a route that proceeded through the unsaturated nucleoside (357).[565]

Two groups have described closely similar syntheses of the amino-acid streptolidine (358), a derivative of 2,3,5-triamino-2,3,5-trideoxy-D-arabinonic acid isolated from roseothricin, from suitably protected derivatives of methyl 2,3,5-triamino-2,3,5-trideoxy-β-D-arabinofuranoside [566, 567] (*cf.* Vol. 3, p. 87).



(358)



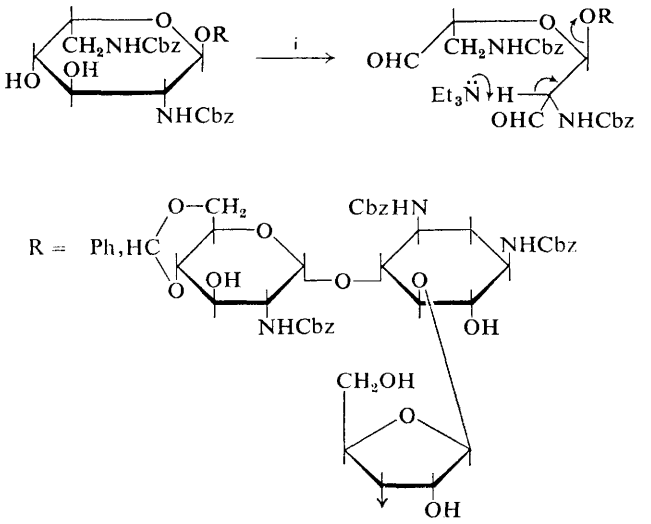
(359)

Many modifications to existing carbohydrate antibiotics have been reported during the past year. 7(*S*)-Alkylated derivatives of lincomycin have been prepared by a route involving alcoholysis of the *N*-acetylepimine (359),[568] and conventional transformations on naturally occurring antibiotics have yielded 3′,4′-deoxy-neamine,[569] 3′,4′-dideoxy-butirosin A,[570] 6′-amino-6′-deoxy-deriva-

[563] E. M. Acton, A. N. Fujiwara, and D. W. Henry, *J. Medicin. Chem.*, 1974, **17**, 659.
[564] S. M. David and J.-C. Fischer, *Carbohydrate Res.*, 1974, **38**, 147.
[565] K. A. Watanabe, T. M. K. Chiu, D. H. Hollenberg, and J. J. Fox, *J. Org. Chem.*, 1974, **39**, 2482.
[566] T. Goto and T. Ohgi, *Tetrahedron Letters*, 1974, 1413.
[567] S. Kusumoto, S. Tsuji, and T. Shiba, *Tetrahedron Letters*, 1974, 1417.
[568] B. Bannister, *J.C.S. Perkin I*, 1974, 360.
[569] H. Saeki, Y. Shimada, N. Takeda, I. Igarashi, S. Sugawara, and E. Ohki, *Sankyo Kenkyusho Nempo*, 1973, **25**, 62 (*Chem. Abs.*, 1974, **80**, 121 240p).
[570] H. Saeki, Y. Shimada, Y. Ohashi, M. Tajima, S. Sugawara, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1145.

tives of lividomycin B,[571] 3′-deoxy-ribostamycin,[572] 5″-amino-5″-deoxy-butirosin,[573] 4″-deoxy-gentamycin C_1,[574] 6′-amino-6′-deoxy-gentamycin A,[575] 2″-epigentamycin C_1, and 2″-deoxy- and 2″-deoxy-3″-des(methylamino)-2″-methylamino-gentamycin C_2.[576]

A derivative of paromomycin has been degraded to give a biologically active pseudo-trisaccharide, 5-*O*-β-D-ribofuranosyl-paromamine, by way of base-catalysed removal of the diaminohexosyl residue following periodate oxidation (Scheme 111).[577] 6-*O*-β-D-Ribofuranosyl-paromamine, an isomeric pseudotri-



Reagent: i, H_5IO_6

Scheme 111

saccharide obtained from paromamine *via* a conventional Koenigs–Knorr reaction, proved to be significantly less active against bacteria; although the 5- and 6-hydroxy-groups of the paromamine derivative were unprotected, only the latter reacted in the condensation reaction.[577a]

Methylation of formycin with methyl iodide occurred at either of the two nitrogen atoms of the pyrazole ring.[578]

Cladinose has been removed selectively from erythromycin A by treating the derived oxime with methanolic hydrogen chloride, whereas desosamine was

[571] I. Watanabe, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 802.
[572] D. Ikeda, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 799.
[573] T. P. Culbertson, D. R. Watson, and T. H. Haskell, *J. Antibiotics*, 1973, **26**, 790.
[574] A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J. Antibiotics*, 1973, **26**, 782.
[575] T. L. Nagabhushan and P. J. L. Daniels, *J. Medicin. Chem.*, 1974, **17**, 1030.
[576] P. J. L. Daniels, J. Weinstein, R. W. Tkach, and J. Morton, *J. Antibiotics*, 1974, **27**, 150.
[577] T. Takamoto and S. Hanessian, *Tetrahedron Letters*, 1974, 4009.
[577a] T. Ogawa, T. Takamoto, and S. Hanessian, *Tetrahedron Letters*, 1974, 4013.
[578] L. B. Townsend, R. A. Long, J. P. McGraw, D. W. Miles, R. K. Robins, and H. Eyring, *J. Org. Chem.*, 1974, **39**, 2023.

simultaneously removed by formation of the *N*-oxide, elimination to form the unsaturated derivative, and methanolysis.[579]

Investigations of the biosynthesis of the mitomycin antibiotics have shown that 2-[^{15}N]amino-2-deoxy-D-[1-^{13}C]glucose is incorporated without breakdown.[580] Related studies on neomycin by Rinehart's group have demonstrated that 2-amino-2-deoxy-D-[1-^{13}C]glucose is incorporated into each of the components with the label at C-1, whereas D-[6-^{13}C]glucose labelled neosamines B and C at C-6, deoxystreptamine at C-2, and D-ribose at C-5.[581] Thus, both D-glucose and 2-amino-2-deoxy-D-glucose are indicated to be specific precursors, with C-1 or C-6 of the precursors becoming either C-1 or C-6, respectively, of the neamines.

Structural investigations on ristosamine and a synthesis of an analogue of daunosamine are noted in Chapter 8, and physical measurements relating to antibiotics are covered in Chapter 24.

[579] R. A. Lemahieu, M. Carson, R. W. Kierstead, L. M. Fern, and E. Grunberg, *J. Medicin. Chem.*, 1974, **17**, 953.

[580] U. Hornemann, J. P. Kehrer, C. S. Nunez, and R. L. Ranieri, *J. Amer. Chem. Soc.*, 1974, **96**, 320.

[581] K. L. Rinehart, jun., J. M. Malik, R. S. Nystrom, R. M. Stroshane, S. T. Truitt, M. Taniguchi, J. P. Rolls, W. J. Haak, and B. A. Ruff, *J. Amer. Chem. Soc.*, 1974, **96**, 2263.

21
Nucleosides

A comprehensive review has dealt with the structures and the functions of nucleosides and nucleotides, including base-pairing and -stacking and the mechanism of action of ribonuclease; mention is also made of the 'rare' nucleosides 4-thiouridine, N^6-isopentenyladenine, and dihydrouridine, and the anti-leukaemic drug 6-azauridine.[582] The chemical synthesis and transformations of nucleosides have also been reviewed,[583] while another review has discussed the evolution of nucleosides (and, hence, nucleic acids) on primitive earth and simulations of the prebiotic formation of sugars and bases in the laboratory.[584]

Two groups have independently identified 3-(3-amino-3-carboxypropyl)uridine as a component of tRNA from *E. coli*.[585, 586] Ezomycins A_1 and A_2, antibiotics containing cytosine linked to an octose derivative,[544] and coformycin, a nucleoside antibiotic containing 1,3-diazepine,[546, 547] are referred to in Chapter 20.

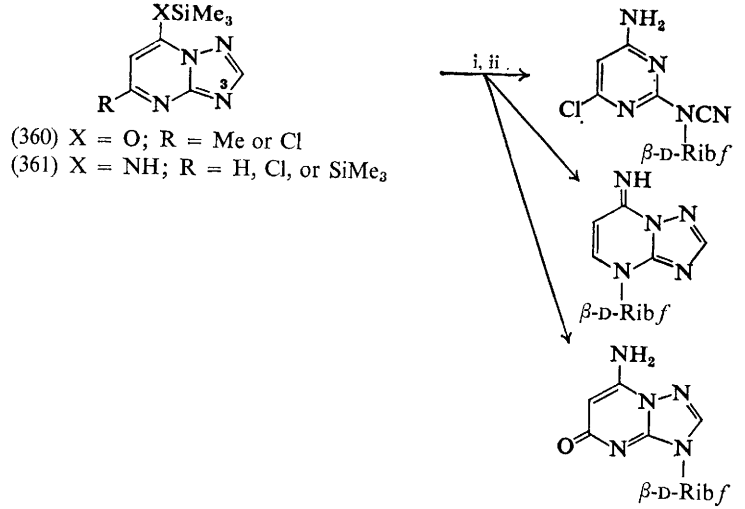
Synthesis

Adenosine and cobalamin have been synthesized from D-[5-^{13}C]ribose.[18] Condensation of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose with N^6-benzoyl- or -benzyl-adenine in nitrophenol occurred at both N-7 and N-9, but the former product rearranged to give the normal N-9 isomer under the conditions used.[587] A number of analogues of 5′-*S*-methyl-5′-thioadenosine, produced by variations of the sugar, the base, and the *S*-alkyl group, have been described.[588] Methyl 5-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-rhamnofuranoside has been converted into 9-α-D-rhamnofuranosyladenine by standard procedures,[589] and 5′-deoxy[590] and carbocyclic[591] analogues of puromycin have been synthesized.

Syntheses have also been reported of the α- or β-D-ribofuranosyl and/or 2-deoxy-β-D-*erythro*-pentofuranosyl derivatives of 4,5,6,7-tetrahydrothiazolo-[4,5-*d*]pyrimidine-5,7-dione (a thio-analogue of 3-isoxanthosine),[592] oxazolo-[5,4-*d*]pyrimidin-7-ones,[593] 5-fluoro-2-methoxypyridines (3-deazauridine deriva-

[582] W. Saenger, *Angew. Chem. Internat. Edn.*, 1973, **12**, 591.
[583] L. Goodman, *Basic Principles of Nucleic Acid Chemistry*, 1974, **1**, 93.
[584] L. E. Orgel and R. Lohrmann, *Accounts Chem. Res.*, 1974, **7**, 368.
[585] Z. Ohashi, M. Maeda, J. A. McCloskey, and S. Nishimura, *Biochemistry*, 1974, **13**, 2620.
[586] S. Friedman, H. J. Li, K. Nakanishi, and G. Van Lear, *Biochemistry*, 1974, **13**, 2932.
[587] N. Nakazaki, M. Sekiya, T. Yoshino, and Y. Ishido, *Bull. Chem. Soc. Japan*, 1973, **46**, 3858.
[588] J. A. Montgomery, A. T. Shortnacy, and H. J. Thomas, *J. Medicin. Chem.*, 1974, **17**, 1197.
[589] L. M. Lerner, *Carbohydrate Res.*, 1974, **38**, 328.
[590] R. G. Almquist and R. Vince, *J. Medicin. Chem.*, 1973, **16**, 1396.
[591] R. Vince and S. Daluge, *J. Medicin. Chem.*, 1974, **17**, 578.
[592] Y. Mizuno, Y. Watanabe, and K. Ikeda, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1198.
[593] V. D. Patil, D. S. Wise, L. B. Townsend, and A. Bloch, *J. Medicin. Chem.*, 1974, **17**, 1282.

tives),[594] 6-selenoguanine,[595] pyridazin-4(1*H*)-ones,[596] isoxanthopterin,[597] and methyl 4(5)-nitroimidazole-5(4)-carboxylates.[598] Glycosylation of the purine analogues (360) resulted in substitution at N-3, whereas the analogues (361) furnished a variety of products (see Scheme 112).[599] Model nucleosides derived



(360) X = O; R = Me or Cl
(361) X = NH; R = H, Cl, or $SiMe_3$

Reagents: i, 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide–$AlCl_3$; ii, NH_3–MeOH

Scheme 112

from D-psicose and guanine have been synthesized in connection with a study of the inhibitory action of psicofuranine; various 1′-deoxy-derivatives were also prepared.[600]

Stannic chloride, a catalyst frequently used in the preparation of nucleosides from acylated sugars and trimethylsilylated pyrimidines, has now been demonstrated to be equally effective in the synthesis of nucleosides from trimethylsilylated purines.[601] A 1,2-acetalated sugar has been used in a new synthesis of β-nucleosides having the 2′-hydroxy-group unsubstituted (Scheme 113).[602] Other ribonucleosides to be synthesized by the silyl procedure include those from 4-pyridone,[603] 2,3-dihydro-1,3-oxazine-2,6-dione,[604] 5-methyl-6-azacytidine,[605] and

[594] S. Nesnow and C. Heidelberger, *J. Heterocyclic Chem.*, 1973, **10**, 779.
[595] G. H. Milne and L. B. Townsend, *J. Medicin. Chem.*, 1974, **17**, 263.
[596] G. L. Szekeres, R. K. Robins, and R. A. Long, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 97.
[597] K. Eistetter and W. Pfleiderer, *Chem. Ber.*, 1974, **107**, 575.
[598] H. Gugljelmi, *Annalen*, 1973, 1286.
[599] G. R. Revankar, R. K. Robins, and R. L. Tolman, *J. Org. Chem.*, 1974, **39**, 1256.
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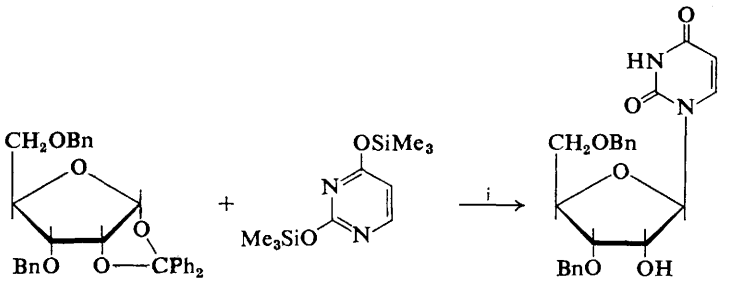
[601] F. W. Lichtenthaler, P. Voss, and A. Heerd, *Tetrahedron Letters*, 1974, 2141.
[602] G. Ritzmann, R. S. Klein, H. Ohrui, and J. J. Fox, *Tetrahedron Letters*, 1974, 1519.
[603] W. J. Woodford, B. A. Swartz, C. J. Pillar, A. Kampf, and M. P. Mertes, *J. Medicin. Chem.*, 1974, **17**, 1027.
[604] T. L. Chwang and C. Heidelberger, *Tetrahedron Letters*, 1974, 95.
[605] H. Hřebabecký and J. Beránek, *Coll. Czech. Chem. Comm.*, 1974, **39**, 976.



Reagent: i, $SnCl_4$–CH_2Cl_2

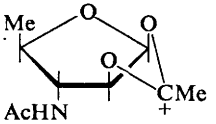
Scheme 113

2′-deoxy-5-ethylcytidine.[606] The silyl procedure was also used in the preparation of derivatives of 1-β-D-xylofuranosyl-thymine and -5-hydroxymethyluracil,[607] 1-β-D-psicofuranosyl-uracil and -cytosine,[608] 6-fluoro-1-β-D-glucofuranosyl-thymine,[609] 1-(5-deoxy-β-D-*ribo*-hexofuranosyl)cytosine,[610] and 4′-thiocytidine (see p. 146).[611] Treatment of trimethylsilylated derivatives of adenine, uracil, and cytosine with 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-4-*O*-methylsulphonyl-α-D-galacto-pyranose afforded β-nucleosides, which were converted by standard procedures into derivatives containing 4-amino-4-deoxy-β-D-glucopyranose.[612] Related syntheses of 1-(4-amino-4-deoxy-β-D-galactopyranosyl)-uracil and -cytosine were described in the accompanying paper.[613] In related work on the synthesis of nucleosides containing hexuronic acid and amino-sugar residues, it was shown that treatment of 1-*O*-acylglycoses with N^4-acetyl-N^4,*O*-bis(trimethylsilyl)-cytosine in the presence of stannic chloride yielded only N-1-substituted β-glyco-sides, whereas a similar reaction with 2,4-bis(trimethylsilyl)uracil gave both N-1- and N-3-substituted β-glycosides; the use of benzoylated sugars favoured glycosylation at N-1.[614] By contrast, the fusion procedure has been used less extensively over the past year, although two reports have suggested that 1,2-acyloxonium ions are intermediates in the formation of nucleosides by fusion. Thus, fusion of a mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α- and -β-D-arabino-furanose with either 2,6-dichloropurine or 7-methylthio-ν-triazolo[4,5-*d*]pyrimid-ine resulted in the formation of α-nucleosides, whereas the pure β-anomer of the acylated sugar was unreactive.[615] Moreover, fusion of a mixture of 3-acetamido-1,2-di-*O*-acetyl-3,5-dideoxy-α- and -β-D-ribofuranoses with 6-chloropurine gave four products, which were separated after conversion into 6-dimethylamino-purine nucleosides; both *arabino* (15%) and *ribo* (25%) isomers were obtained,

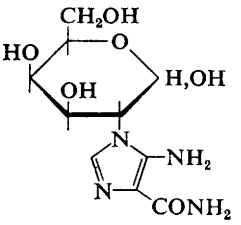
[606] T. Kulikowski and D. Shugar, *J. Medicin. Chem.*, 1974, **17**, 269.
[607] N. N. Artem'eva, B. N. Stepanenko, and E. M. Kaz'mina, *Khim.-Farm. Zhur.*, 1974, **8**, 26 (*Chem. Abs.*, 1974, **80**, 121 250s).
[608] H. Hřebabecký and J. Farkaš, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1098.
[609] D. Bärwolff, G. Kowollik, and P. Langen, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1494.
[610] S. David and G. De Sennyey, *Compt. rend.*, 1974, **279**, *C*, 651.
[611] N. Ototani and R. L. Whistler, *J. Medicin. Chem.*, 1974, **17**, 535.
[612] F. W. Lichtenthaler, P. Voss, and G. Bambach, *Bull. Chem. Soc. Japan*, 1974, **47**, 2297.
[613] F. W. Lichtenthaler, T. Ueno, and P. Voss, *Bull. Chem. Soc. Japan*, 1974, **47**, 2304.
[614] F. W. Lichtenthaler, A. Heerd, and K. Strobel, *Chem. Letters*, 1974, 449.
[615] D. A. Baker, R. A. Harder, jun., and R. L. Tolman, *J.C.S. Chem. Comm.*, 1974, 167.

the former presumably arising by way of an acetoxonium-ion intermediate (362).[616] A series of pteridine nucleosides was also obtained by the fusion procedure.[617]

Preobrazhenskaya's group has prepared a number of analogues of purine nucleosides, mostly by condensation of a suitably protected sugar with an appropriate heterocyclic derivative; these include 5(6)-fluoro-1-D-ribofuranosylindolines,[618] 1-β-D-glucopyranosyl-pyrrolo[2,3-*b*]pyridine[619] and -indole,[620]



(362)



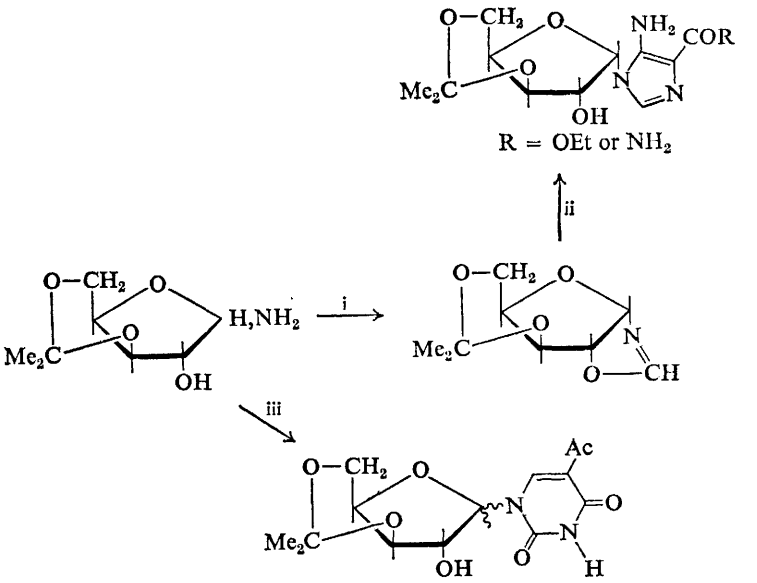
(363)

3-chloro-1-D-xylofuranosylindazoles[621] and the corresponding derivative of β-D-ribofuranose,[621a] and D-ribofuranosyl and D-glucopyranosyl derivatives of pyrazolo[3,4-*b*]pyrazine.[622] By contrast, 1-β-D-glucopyranosylisatin derivatives were prepared by treatment of the corresponding glycosylaniline derivative with oxalyl chloride.[623]

Glycosylamine derivatives of D-xylose, D-glucose, D-mannose, and L-rhamnose have been converted into imidazole and pyrimidine nucleosides by the route shown in Scheme 114.[624, 625] A similar route was used to prepare such 'reducing nucleosides' as (363), having imidazole linked to either C-2 or C-3 of the sugar residue.[626]

Treatment of various uracil and uridine derivatives with chlorothiocyanogen afforded 5-thiocyanato derivatives, which could be reduced to the corresponding

[616] R. Vince and R. G. Almquist, *Carbohydrate Res.*, 1974, **36**, 214.
[617] M. Ott and W. Pfleiderer, *Chem. Ber.*, 1974, **107**, 339.
[618] V. I. Mukhanov, M. N. Preobrazhenskaya, N. P. Kostyuchenko, T. Ya. Filipenko, and N. N. Suvorov, *Zhur. org. Khim.*, 1974, **10**, 587.
[619] M. N. Preobrazhenskaya, T. D. Miniker, V. S. Martynov, L. N. Yakhon Tov, N. P. Kostyuchenko, and D. M. Kranokutskaya, *Zhur. org. Khim.*, 1974, **10**, 745.
[620] M. N. Preobrazhenskaya, Yu. A. Zhdanov, V. P. Shabunova, and N. N. Suvorov, *Zhur. org. Khim.*, 1973, **9**, 2624.
[621] I. A. Korbukh, L. N. Abramova, B. N. Stepanenko, and M. N. Preobrazhenskaya, *Doklady Akad. Nauk S.S.S.R.*, 1974, **216**, 564.
[621a] I. A. Korbukh, F. F. Blanko, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1974, **10**, 1091.
[622] I. A. Korbukh, M. N. Preobrazhenskaya, H. Dorn, N. G. Kondakova, and N. P. Kostyuchenko, *Zhur. org. Khim.*, 1974, **10**, 1095.
[623] M. N. Preobrazhenskaya, I. V. Yartseva, and L. V. Ektova, *Doklady Akad. Nauk S.S.S.R.*, 1974, **215**, 873.
[624] N. J. Cusack, D. H. Robinson, P. W. Rugg, G. Shaw, and R. Lofthouse, *J.C.S. Perkin I*, 1974, 73.
[625] D. H. Robinson and G. Shaw, *J.C.S. Perkin I*, 1974, 774.
[626] D. V. Wilson and C. G. Beddows, *Experientia*, 1974, **30**, 588.



Reagents: i, NH:CHOEt; ii, $H_2NCH(CN)COR$; iii, $EtOCH{:}C(Ac)CONHCO_2Et$

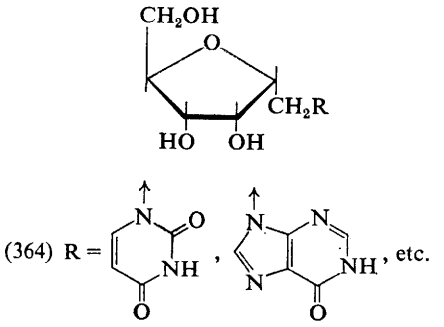
Scheme 114

5-thiols.[627] Pyrrolo[2,3-*d*]pyrimidine nucleosides have been converted into fluorescent imidazo[1,2-*c*]pyrrolo[2,3-*d*]pyrimidine derivatives by the action of chloroacetaldehyde.[628]

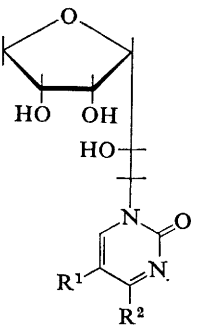
'Reversed' Nucleosides and 'Homonucleosides'

'Reversed' nucleosides containing uracil and adenine linked to C-6 of methyl α-D-glucopyranoside have been reported.[629] 'Double-headed' nucleosides containing either indol-1-yl or carbazol-9-yl residues attached to C-5′ of uridine and azauridine have been obtained by a displacement reaction on appropriate nucleoside 5′-toluene-*p*-sulphonates with the sodium salt of either indole or carbazole.[378a, 630] Reduction of 2,3,5-tri-*O*-benzyl-α- and -β-D-ribofuranosyl cyanides with lithium aluminium hydride gave a separable mixture of benzylated 1-amino-2,5-anhydro-1-deoxy-D-altritol and -D-allitol, and the former isomer was converted into the α-homonucleosides (364).[397] 3,6-Anhydro-2-*O*-benzoyl-4,5-*O*-isopropylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol has been used in syntheses of related homonucleosides, *e.g.* (365), of uracil, thymine, and cytosine.[631]

[627] T. Nagamachi, J.-L. Fourrey, P. F. Torrence, J. A. Waters, and B. Witkop, *J. Medicin. Chem.*, 1974, **17**, 403.
[628] K. H. Schram and L. B. Townsend, *Tetrahedron Letters*, 1974, 1345.
[629] N. Ueda, Y. Nakatani, S. Terada, K. Kondo, and K. Takemoto, *Technol. Rep. Osaka Univ.*, 1973, **23**, 713 (*Chem. Abs.*, 1974, **81**, 49 969j).
[630] M. N. Preobrazhenskaya, S. Ya. Mel'nik, E. A. Utkina, E. G. Sokolova, and N. N. Suvorov, *Zhur. org. Khim.*, 1974, **10**, 863.
[631] V. Zecchi, L. Garuti, G. Giovanninetti, L. Rodriguez, M. Amorosa, and J. Defaye, *Bull. Soc. chim. France*, 1974, 1389.



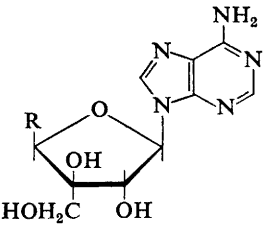
(364) R =



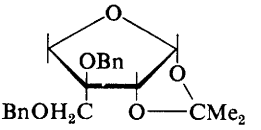
(365) R^1 = H; R^2 = NHAc
R^1 = Me; R^2 = OH

Nucleosides with Branched-chain Components

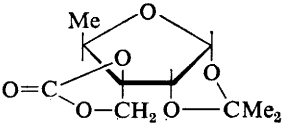
The branched-chain adenine nucleosides (366)[632] and (367)[633] have been synthesized from the 1,2-*O*-isopropylidene derivatives (368) and (369) by standard procedures; in contrast to the C-3′ epimers previously synthesized, both (366)



(366) R = H
(367) R = Me

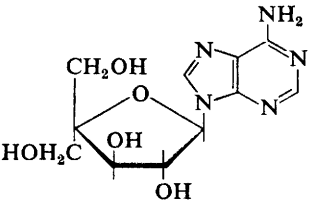


(368)

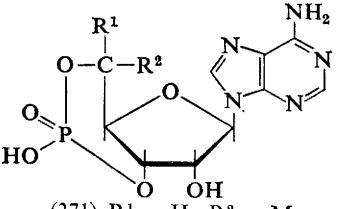


(369)

and (367) underwent enzymic deamination. A synthesis of the 4′-*C*-branched adenine nucleoside (370) has been described.[470] The addition of Grignard reagents to 5′-aldehydo and 5′-uronate ester derivatives of adenosine has furnished 5′-mono- and 5′-di-*C*-methyl analogues of adenosine, which were

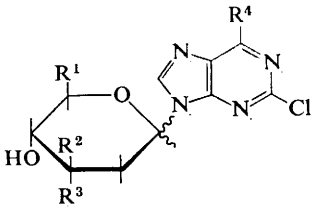


(370)



(371) R^1 = H; R^2 = Me
(372) R^1 = R^2 = Me

[632] J. M. J. Tronchet and J. Tronchet, *Carbohydrate Res.*, 1974, **34**, 263.
[633] J. M. J. Tronchet, J. Tronchet, and R. Graf, *J. Medicin. Chem.*, 1974, **17**, 1055.

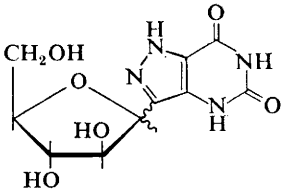


(373) $R^1 = R^3 = H$; $R^2 = CH_2CN$; $R^4 = Cl$
(374) $R^1 = CH_2OH$; $R^2 = H$; $R^3 = CH_2CN$; $R^4 = NMe_2$

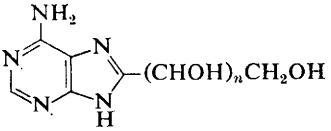
converted into the 3′,5′-cyclic phosphates (371) and (372).[634] The 3′-*C*-cyanomethyl nucleosides (373) and (374) have been prepared using Wittig reactions to introduce the chain branch.[444, 445]

C-Nucleosides

The α- and β-D-*arabino* analogues (375) of oxoformycin B have been elaborated from 2,3,5-tri-*O*-benzyl-α- and -β-D-arabinofuranosyl cyanides by reduction of the nitrile group with diborane followed by construction of the heterocyclic system *via* dipolar addition to a derived 1-diazo-sugar.[635] In continuing their work on the synthesis of *C*-analogues of *N*-nucleoside antibiotics, El Khadem



(375)



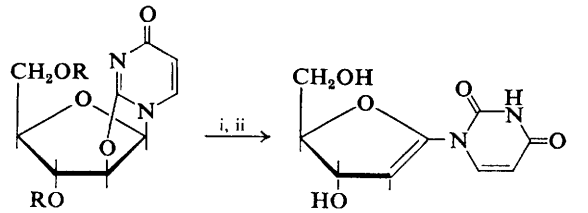
(376)

et al. have reported syntheses of 8-β-D-arabinofuranosyladenine,[636] 6-amino-8-(3-deoxy-β-D-*erythro*-pentofuranosyl)purine (a *C*-analogue of cordycepin, see Vol. 7, p. 158),[637] and the 8-(hydroxyalkyl)adenines (376), obtained by condensing 4,5,6-triaminopyrimidine with aldonic acids of various chain-lengths and pyrolysis of the resulting amides.[638] A number of related compounds are noted in Chapters 2 and 3.

Unsaturated Nucleosides

Of special interest have been the first syntheses of 1′,2′-unsaturated pyrimidine[639] and purine[640] nucleosides by Robins' group (see Schemes 115 and 116). The unsaturated-sugar nucleoside derived from uracil was extremely sensitive to acids,

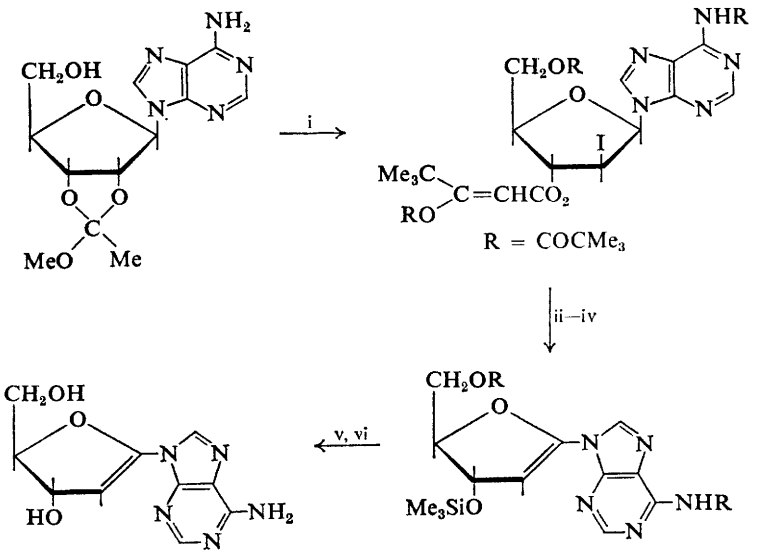
[634] R. S. Ranganathan, G. H. Jones, and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 290.
[635] E. M. Acton, A. N. Fujiwara, L. Goodman, and D. W. Henry, *Carbohydrate Res.*, 1974, **33**, 135.
[636] H. S. El Khadem and D. L. Swartz, *Carbohydrate Res.*, 1974, **32**, C1.
[637] H. S. El Khadem and E. S. H. El Ashry, *Carbohydrate Res.*, 1974, **32**, 339.
[638] H. S. El Khadem and R. Sindric, *Carbohydrate Res.*, 1974, **34**, 203.
[639] M. J. Robins and E. M. Trip, *Tetrahedron Letters*, 1974, 3369.
[640] M. J. Robins and R. A. Jones, *J. Org. Chem.*, 1974, **39**, 113.



Reagents: i, $KOBu^t$–DMF; ii, $Et_4N^+F^-$

Scheme 115

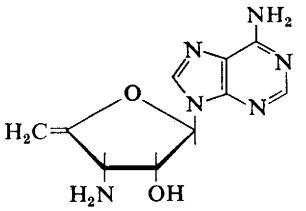
decomposed to uracil on storage, and gave equal proportions of the α- and β-forms of 2′-deoxyuridine on hydrogenation over palladized carbon. 3′-Amino-3′-deoxyadenosine reacted with phosphorus oxychloride in triethyl phosphate to give either the 5′-chloro-5′-deoxy derivative or a mixture of the 5′-mono- and 2′,5′-di-phosphates, depending on the conditions; dehydrochlorination of the chlorinated derivative with potassium t-butoxide gave a new analogue (377) of augustomycin A.[641] A related nucleoside (378) was obtained during the course of investigations on fluoro-sugar nucleosides[642] (see also p. 150). Other examples of unsaturated nucleosides are noted in Chapter 14.



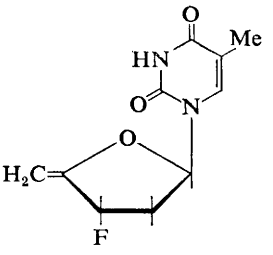
Reagents: i, Me_3CCOCl–py–NaI; ii, $KMnO_4$; iii, Me_3SiCl–py; iv, DBN; v, MeOH–H^+; vi, MeOH–MeONa

Scheme 116

[641] M. Morr and M.-R. Kula, *Tetrahedron Letters*, 1974, 23.
[642] G. Kowollik, G. Etzold, M. Von Janta-Lipinski, K. Gaertner, and P. Langen, *J. prakt. Chem.*, 1973, **315**, 895.



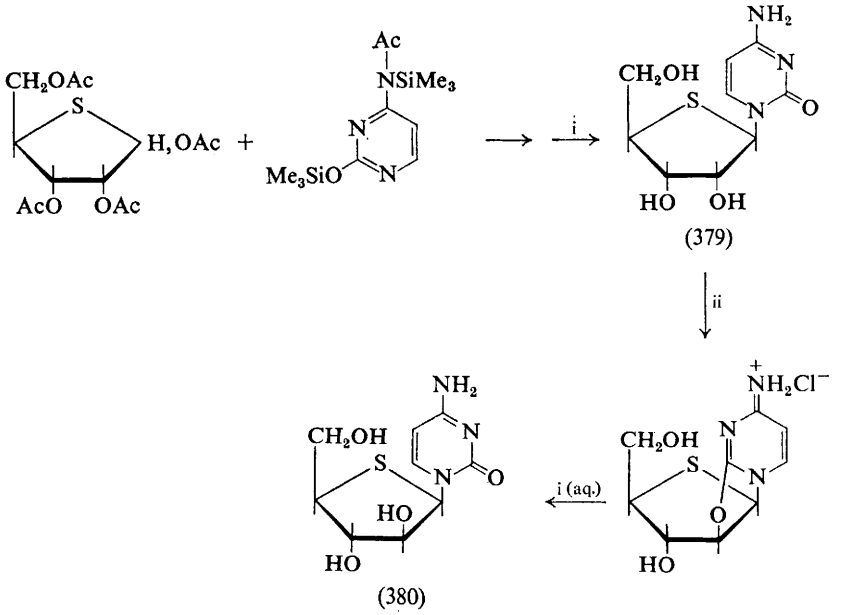
(377)



(378)

Cyclonucleosides

2,2′-Cyclonucleosides have been used as intermediates in the conversion of D-ribonucleosides into D-arabinonucleosides,[643] a process simplified by the one-step conversion of pyrimidine nucleosides into 2,2′-cyclonucleosides with phosphorus oxychloride in DMF. This process is exemplified by the conversion of 4′-thiocytidine (379) into the D-thioarabinonucleoside (380) (Scheme 117).[611]



(379)

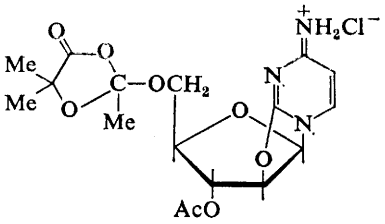
(380)

Reagents: i, NH_3; ii, $POCl_3$–DMF

Scheme 117

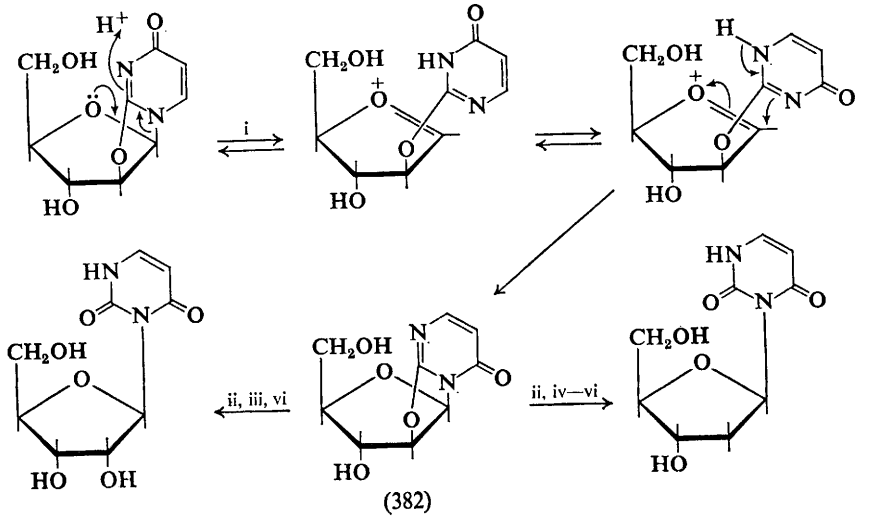
2-Acetoxyisobutyryl chloride has also been used to prepare 2,2′-cyclonucleosides; thus, the initial product (381) from the reaction with cytidine afforded 2,2′-anhydrocytidine (75%) following treatment with methanolic hydrogen chloride

[643] T. Kanai, M. Ichino, A. Hoshi, F. Kanzawa, and K. Kuretani, *J. Medicin. Chem.*, 1974, **17**, 1076.



(381)

and deacetylation.[644] 2,2′-Anhydrocytidine showed pronounced anti-viral activity. Reactions similar to those noted above were used to convert (381) into 1-β-D-arabinofuranosylcytosine. A dinucleotide containing uridine and a D-arabino-nucleoside has been synthesized from 2,2′-anhydrouridine.[645] 2,2′-Anhydro-uridine rearranged in liquid hydrogen fluoride by cleavage of the N-1–glycosyl linkage followed by formation of an N-3–glycosyl linkage; the rearranged product (382) was used to prepare other nucleosides, as shown in Scheme 118.[646]



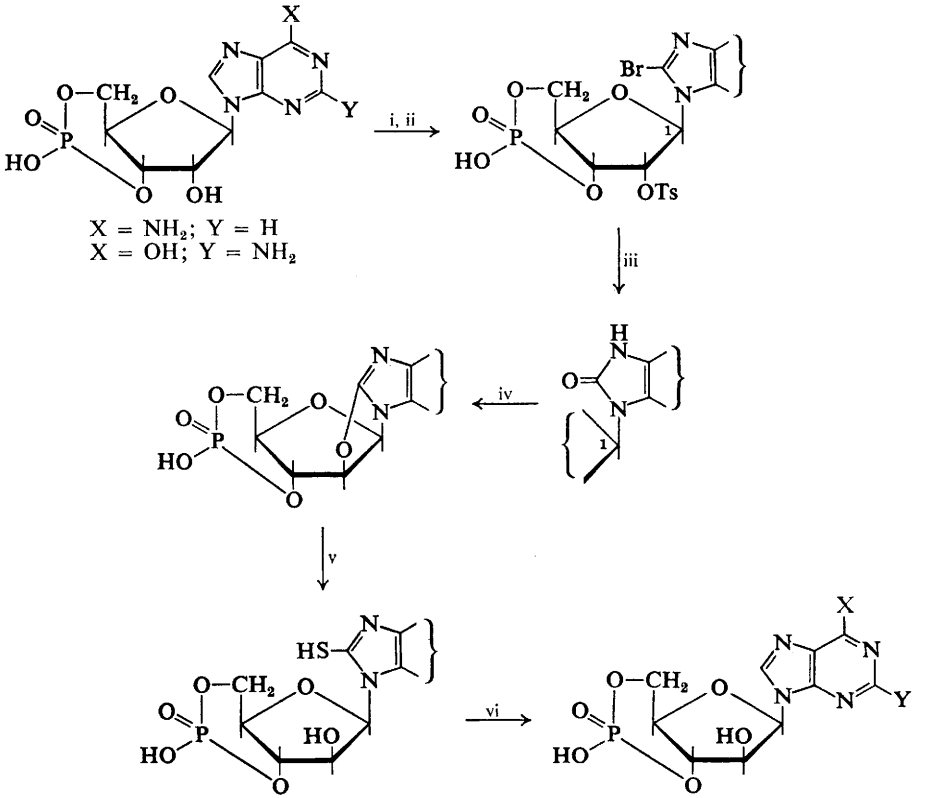
(382)

Reagents: i, HF; ii, BzCl–py; iii, BF_3–MeOH; iv, HCl–DMF; v, Bu_3SnH; vi, MeOH–MeONa

Scheme 118

The *ribo* into *arabino* conversion has also been effected with the 3′,5′-cyclic phosphates of adenosine and guanosine by way of the 8,2′-anhydronucleosides (Scheme 119).[647] 8,5′-Anhydro-2′,3′-*O*-isopropylideneadenosine has been shown to rearrange to the corresponding N^3,5′-anhydronucleoside on heating with

[644] A. F. Russell, M. Prystasz, E. K. Hamamura, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 2182.
[645] K. K. Ogilvie and D. J. Iwacha, *Canad. J. Chem.*, 1974, **52**, 1787.
[646] J. O. Polazzi, D. L. Leland, and M. P. Kotick, *J. Org. Chem.*, 1974, **39**, 3114.
[647] A. M. Mian, R. Harris, R. W. Sidwell, R. K. Robins, and T. A. Khwaja, *J. Medicin. Chem.*, 1974, **17**, 259.

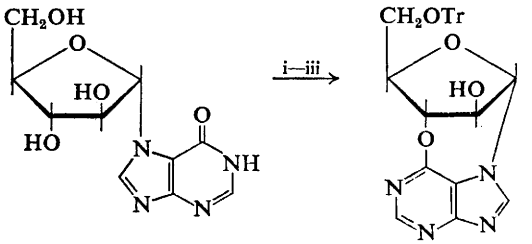


Reagents: i, Br_2; ii, TsCl–py; iii, AcOH–NaOAc; iv, NaOAc–DMF; v, H_2S; vi, Raney Ni

Scheme 119

sodium chloride in DMSO, presumably *via* a 5′-chloro-5′-deoxy-derivative.[648] The synthesis of a new type of purine cyclonucleoside is shown in Scheme 120.[649]

The isomeric 2,2′-, 2,3′-, and 2,5′-thioanhydrouridines have been prepared by standard methods from appropriate sulphonates of 2-thiouridine, and their

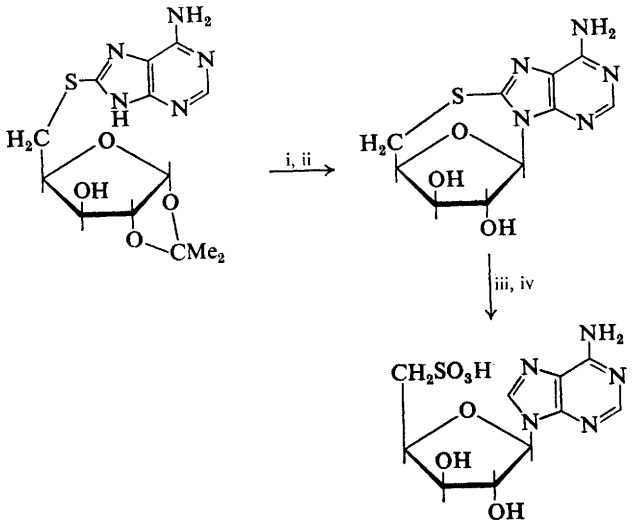


Reagents: i, TrCl–py; ii, MsCl–py; iii, NaOEt

Scheme 120

[648] M. Ikehara and S. Tanaka, *Tetrahedron Letters*, 1974, 497.
[649] Y. Mizuno, Y. Watanabe, K. Ikeda, and J. A. McCloskey, *Heterocycles*, 1974, **2**, 439.

spectroscopic properties have been recorded.[650] Acid-catalysed ring-closure of C-substituted 5-thiopentofuranosyl derivatives of adenine has afforded 8,5′-thioanhydronucleosides of D-arabinose, D-ribose, and D-xylose; oxidation of the D-*xylo*-compound with chlorine gave, after reductive removal of the chloro-group from C-8, a sulphonic acid analogue of 9-β-D-xylofuranosyladenine 5′-phosphate (Scheme 121).[651]



Reagents: i, AcOH–Ac_2O–H_2SO_4; ii, NH_3–MeOH; iii, Cl_2–MeOH–HCl; iv, H_2–Pd/C

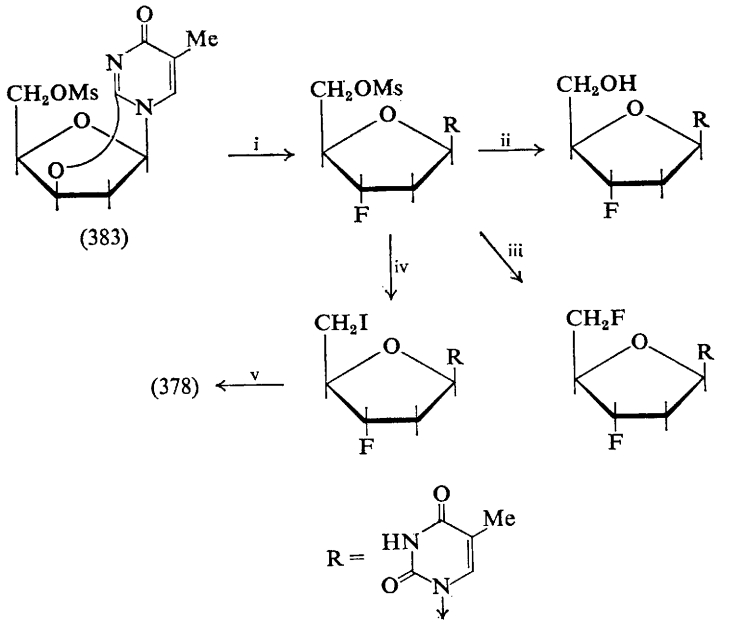
Scheme 121

Cyclonucleosides have also found use in the synthesis of halogeno-sugar nucleosides (see next section).

Halogeno-sugar Nucleosides

The one-step bromination of uridine with acetyl bromide in either acetonitrile or ethyl acetate gave 3′,5′-di-*O*-acetyl-2′-bromo-2′-deoxyuridine in good yield, the reaction proceeding by way of 3′,5′-di-*O*-acetyl-2,2′-anhydrouridine formed from an intermediate 2′,3′-acetoxonium ion.[652] Similar treatment of cytidine gave only the 2,2′-anhydronucleoside, whereas N^4-acetylcytidine furnished N^4-acetyl-1-(2,5-di-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)cytosine by reaction of the initially formed 2′,3′-acetoxonium ion with bromide ions rather than with the cytosine moiety. A similar approach has been adopted by Czech workers, who treated 2,2′-anhydro-3′,5′-di-*O*-benzoylnucleosides with hydrogen chloride to give 2′-chloro-2′-deoxynucleosides, which were reductively dechlorinated to 2′-deoxynucleosides with tri-n-butyltin hydride.[653] A cyclonucleoside (383) was also used as the starting material for a synthesis of a number of nucleosides containing fluoro-sugar residues (Scheme 122).[642]

[650] T. Ueda and S. Shibuya, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 930.
[651] Y. Mizuno, C. Kaneko, and Y. Oikawa, *J. Org. Chem.*, 1974, **39**, 1440.
[652] R. Marumoto and M. Honjo, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 129.
[653] A. Holý and D. Cech, *Coll. Czech. Chem. Comm.*, 1974, **39**, 3157.

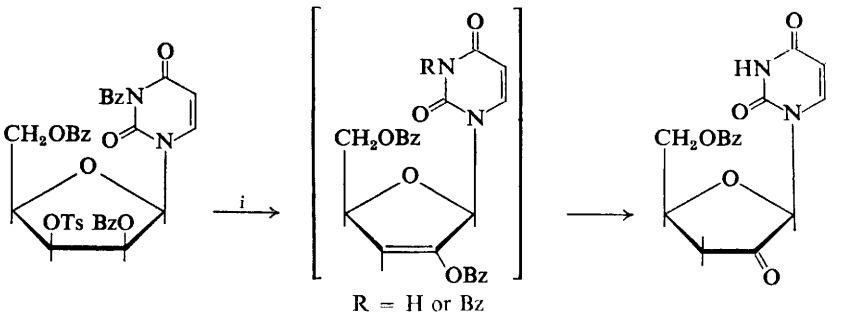


Reagents: i, $HF-AlF_3$; ii, NaOEt–EtOH; iii, $Bu_4N^+F^-$; iv, NaI; v, AgF

Scheme 122

Ketonucleosides and Nucleoside Carboxylic Acids

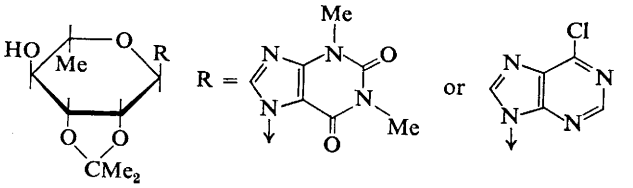
Several routes to 1-(3-deoxy-β-D-*glycero*-pentofuranosyl-2-ulose)uracil and the 5′-benzoate thereof have been reported (*e.g.* Scheme 123).[654] Oxidation of the protected L-rhamnopyranosylnucleosides (384) to the 4′-ketones was achieved with the Pfitzner–Moffatt reagent, whereas oxidation with acetic anhydride–DMSO yielded only the methylthiomethyl ethers.[655] The ketonucleoside (385)



Reagent: i, NaOBz–DMF

Scheme 123

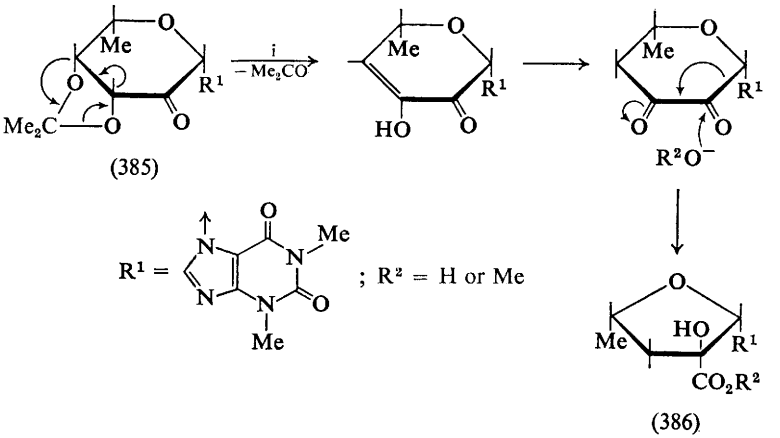
[654] T. Sasaki, K. Minamoto, and K. Hattori, *Tetrahedron*, 1974, **30**, 2689.
[655] J. Herscovici and K. Antonakis, *J.C.S. Perkin I*, 1974, 979.



(384)

reacted with bases to give the isosaccharinic acid derivatives (386) by the mechanism illustrated in Scheme 124.[656]

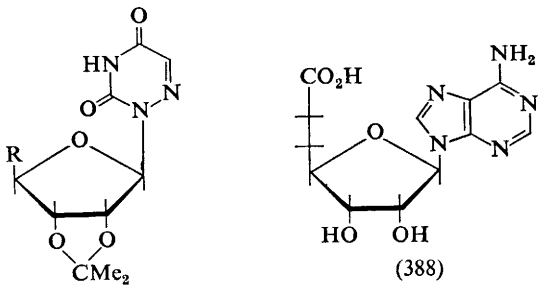
Oxidation of 6-aza-2′,3′-*O*-isopropylideneuridine with sodium periodate in aqueous acetone containing ruthenium trichloride gave the 5′-uronic acid derivative (387), but removal of the isopropylidene group required prolonged



(385) (386)

Reagent: i, NaOH–MeOH

Scheme 124



(387) R = CO_2H
(389) R = $CH_2OCH_2CO_2H$

(388)

[656] T. Halmos, J. Herscovici, and K. Antonakis, *Compt. rend.*, 1974, **279**, *C*, 855.

hydrolysis with hot, aqueous acetic acid.[657] Treatment of 9-(5,6-dideoxy-β-D-*ribo*-heptofuranuronosyl)adenine (388) with DCC yielded dimers (analogues of dinucleotides) that exhibited base-stacking phenomena.[658] A number of nucleoside 5′-uronic acids have been converted, *via* the 5′-uronamides, into 5′-nitriles, which were transformed into 5′-tetrazole derivatives on reaction with ammonium azide.[659]

Other Derivatives

Cytidine reacted with ethyl iodide to give a mixture of products including 3- and N^4-mono-, 3,N^4- and N^4,N^4-di-, and, possibly, 3,N^4,N^4-tri-ethylcytidines.[660] Ethylation of adenosine using either diethyl sulphate or ethyl methanesulphonate in neutral, aqueous solution gave 1-, N^6-, and 7-ethyladenosines.[661] Treatment of purine nucleosides with pivalic acid in the presence of ferrous ions resulted in the methylation of guanine residues at N-8 and adenine and hypoxanthine residues at N-2 or N-8 or both positions.[662]

Mono-*O*-methylation of the secondary hydroxy-groups of unprotected nucleosides can be effected using diazomethane in the presence of catalytic amounts of stannous chloride; purine nucleosides gave the 2′- and 3′-*O*-methyl ethers, with the latter predominating, whereas pyrimidine nucleosides gave mainly the 2′-*O*-methyl ethers.[663] The Hilbert method has been used to synthesize 1-(2-*O*-methyl-β-D-arabinofuranosyl)cytosine from the monomethylated pentose,[664] and the 5′-carboxymethyl ether of 6-azauridine (389) has been prepared.[657] D-Glucopyranosides of the 5-hydroxymethyl derivatives of uracil, 1,3-dimethyluracil, uridine, and 2′-deoxyuridine have been reported.[665, 665a]

Two reports have described the use of bulky silylating reagents in the selective etherification of nucleosides. Thus, with t-butyldimethyl and tri-isopropyl silylating agents, reaction occurred preferentially at O-5′, whereas prolonged reaction afforded mixtures of 2′,5′- and 3′,5′-disilyl ethers from which the 2′- and 3′-silyl ethers could be obtained by utilizing the greater ease of cleavage of the 5′-silyl ether.[666] The investigation was extended to include the silylation of 2′-deoxynucleosides and the use of other bulky silylating reagents, some of which (*e.g.* t-butyltetramethylenesilyl chloride) reacted only at primary hydroxy-groups.[667]

2′,3′-*O*-Dibutylstannylene derivatives of nucleosides are mentioned in Chapter 18.

[657] H. Pischel, A. Holý, and G. Wagner, *Coll. Czech. Chem. Comm.*, 1974, **39**, 3773.
[658] H. Follman, *Angew. Chem. Internat. Edn.*, 1974, **13**, 77.
[659] J. J. Baker, A. M. Mian, and J. R. Tittensor, *Tetrahedron*, 1974, **30**, 2939.
[660] L. Sun and B. Singer, *Biochemistry*, 1974, **13**, 1905.
[661] B. Singer, L. Sun, and H. Fraenkel-Conrat, *Biochemistry*, 1974, **13**, 1913.
[662] M. Maeda, K. Nushi, and Y. Kawazoe, *Tetrahedron*, 1974, **30**, 2677.
[663] M. J. Robins, S. R. Naik, and A. S. K. Lee, *J. Org. Chem.*, 1974, **39**, 1891.
[664] J. A. Montgomery and A. G. Laseter, *J. Medicin. Chem.*, 1974, **17**, 360.
[665] R. Brossmer and V. Eschenfelder, *Annalen*, 1974, 967.
[665a] R. Brossmer and V. Eschenfelder, *Annalen*, 1974, 975.
[666] K. K. Ogilvie, K. L. Sadana, E. A. Thompson, M. A. Quilliam, and J. B. Westmore, *Tetrahedron Letters*, 1974, 2861.
[667] K. K. Ogilvie, E. A. Thompson, M. A. Quilliam, and J. B. Westmore, *Tetrahedron Letters*, 1974, 2865.

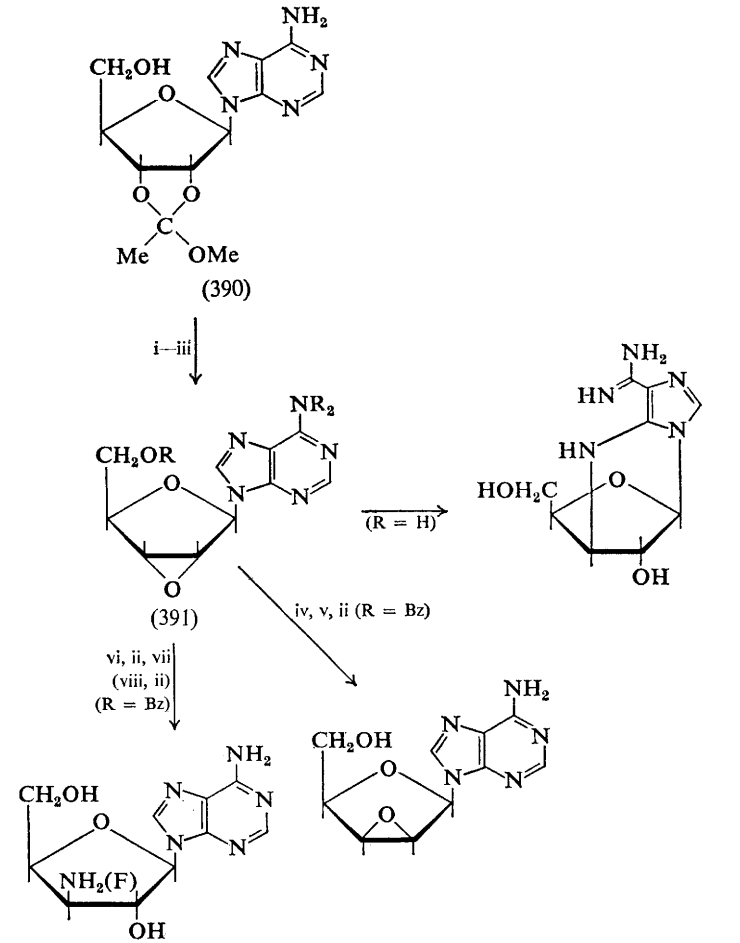
A method for the selective removal of 3′-*O*-acetyl groups from acetylated mono- and oligo-nucleotides has been reported.[668] Syntheses of 3′(2′)-*O*-aminoacyl-2′(3′)-*O*-methyladenosines containing L-leucine, L-phenylalanine, and L-methionine as amino-acid components have been described; 2′-*O*-methyl-3′-*O*-phenylalanyladenosine exhibited biological activity similar to that of puromycin.[669] The aminoacylnucleosides are related to the 3′-terminal units of tRNA, and related work on 3′(2′)-aminoacyl-2′(3′)-deoxyadenosines has been reported.[670]

Selective phosphorylation of 5′-hydroxy-groups in the presence of secondary hydroxy-groups occurred when nucleosides reacted with the *N*-alkylpyridinium salts of five-membered, cyclic acyl phosphates; 2′-deoxynucleoside 5′-phosphates and 3′,5′-cyclic phosphates have been prepared by this procedure.[258a] Tris-(quinol-8-yl) phosphate has been used to prepare nucleoside 5′-bis(quinol-8-yl) phosphates from which the quinolyl groups were removed by treatment with hot, aqueous cupric chloride.[671, 672] 5′-Nucleotide analogues have been prepared from 5′-amino-5′-deoxyguanosine,[673] and a 3′,5′-cyclic phosphoramidate has been derived from 3′-amino-3′-deoxyadenosine 5′-phosphate by cyclization with DCC.[674] Related analogues of nucleotides have been synthesized from 3′(5′)-amino-3′(5′)-deoxythymidine,[332] and syntheses of adenosine 3′,5′-cyclic phosphorothioate[675] and an 8-selenopurine nucleoside 3′,5′-cyclic phosphate[676] have been reported. Nucleoside phosphites have been protected by trimethylsilylation before conversion into either phosphates or thiophosphates by various methods,[677] and uridine 5′-diphenylphosphate has been obtained by appropriate phosphorylation of a polymer-bound nucleoside[218] (see Chapter 5).

Nucleoside 5′-nitrates are noted in Chapter 6.

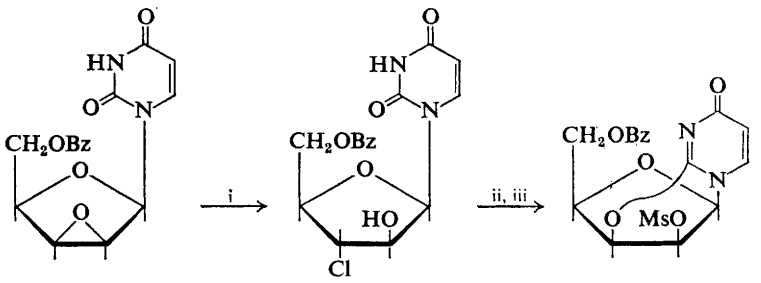
2′,3′-Anhydroadenosine (391; R = H), which has become readily available by reaction of the orthoester (390) with pivaloyl chloride, decomposed spontaneously in water, but the tribenzoylated derivative (391; R = Bz) was readily converted into 3-substituted D-xylonucleosides and the 2′,3′-anhydro-*lyxo*-isomer (see Scheme 125).[678] Regiospecific opening of 1-(2,3-anhydro-5-*O*-benzoyl-β-D-lyxofuranosyl)uracil with pyridinium hydrochloride occurred as shown in Scheme 126.[679] 2′,3′-Anhydro-derivatives were also encountered during conversions of the *C*-nucleoside (392) into various derivatives containing halogeno- and amino-sugars.[680]

[668] Yu. V. Tumanov, V. K. Potapov, M. N. Kochetkova, S. I. Turkin, M. V. Budanov, and Z. A. Shabarova, *Zhur. obshchei Khim.*, 1974, **44**, 1216.
[669] M. J. Robins, M. MacCoss, and G. Ramani, *Canad. J. Chem.*, 1974, **52**, 3803.
[670] M. J. Robins, R. A. Jones, and M. MacCoss, *Biochemistry*, 1974, **13**, 553.
[671] H. Takaku and Y. Shimada, *Tetrahedron Letters*, 1974, 1279.
[672] H. Takaku and Y. Shimada, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1743.
[673] K. Schattka and B. Jastorff, *Chem. Ber.*, 1974, **107**, 3043.
[674] M. Morr, M.-R. Kula, G. Roesler, and B. Jastorff, *Angew. Chem. Internat. Edn.*, 1974, **13**, 280.
[675] F. Eckstein, L. P. Simonson, and H.-P. Baer, *Biochemistry*, 1974, **13**, 3806.
[676] S.-H. Chu, C.-Y. Shiue, and M.-Y. Chu, *J. Medicin. Chem.*, 1974, **17**, 406.
[677] T. Hata and M. Sekine, *Tetrahedron Letters*, 1974, 3943.
[678] M. J. Robins, Y. Fouron, and R. Mengel, *J. Org. Chem.*, 1974, **39**, 1564.
[679] T. Sasaki, K. Minamoto, and N. Kidokoro, *Org. Prep. Proc. Internat.*, 1973, **5**, 75 (*Chem. Abs.*, 1973, **79**, 66 721y).
[680] F. Nouaille, A.-M. Sepulchre, G. Lukacs, A. Kornprobst, and S. D. Gero, *Bull. Soc. chim. France*, 1974, 143.



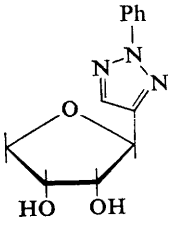
Reagents: i, Me_3CCOCl–py; ii, NaOMe–MeOH; iii, BzCl–py; iv, NaOBz–DMF; v, MsCl–py; vi, NaN_3–DMF; vii, H_2–Pd–C; viii, $Et_4N^+F^-$

Scheme 125



Reagents: i, pyH^+Cl^-; ii, MsCl–py; iii, NaOAc

Scheme 126



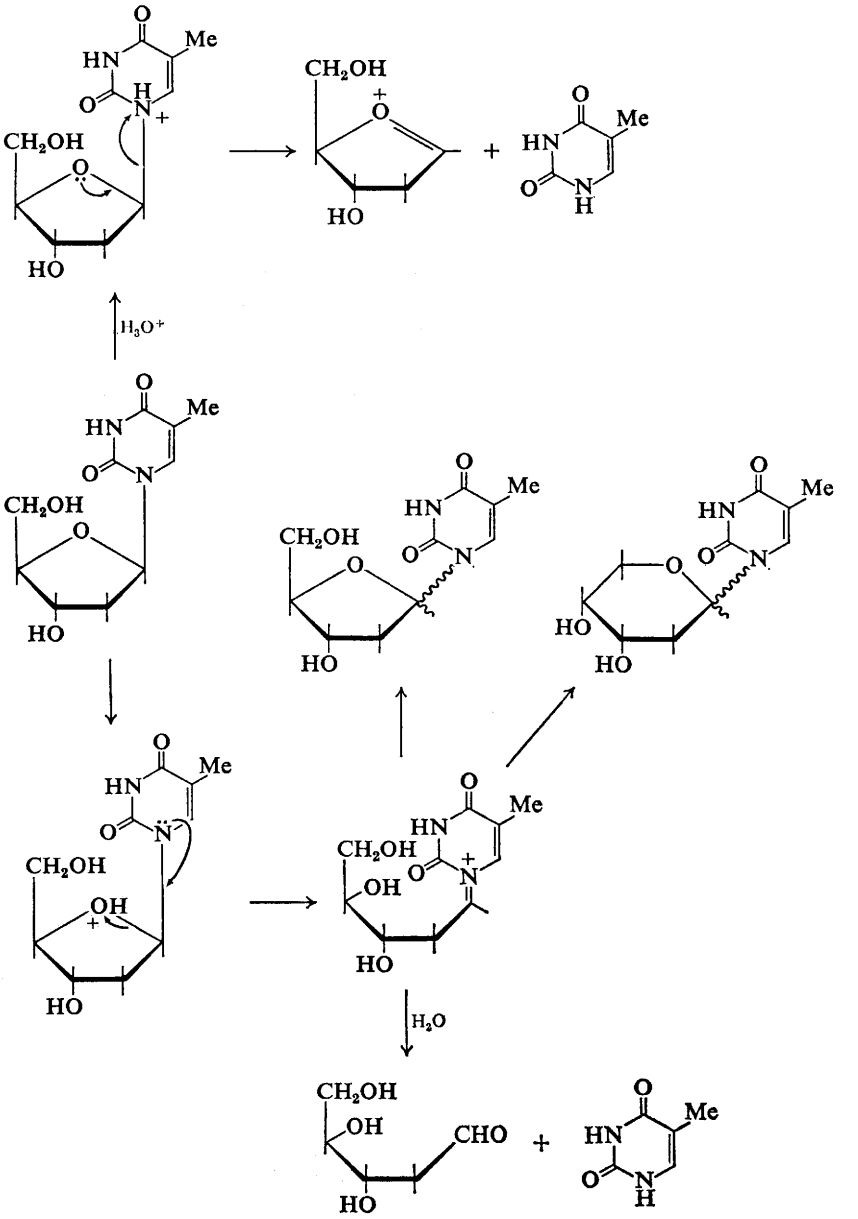
(392)

Reactions

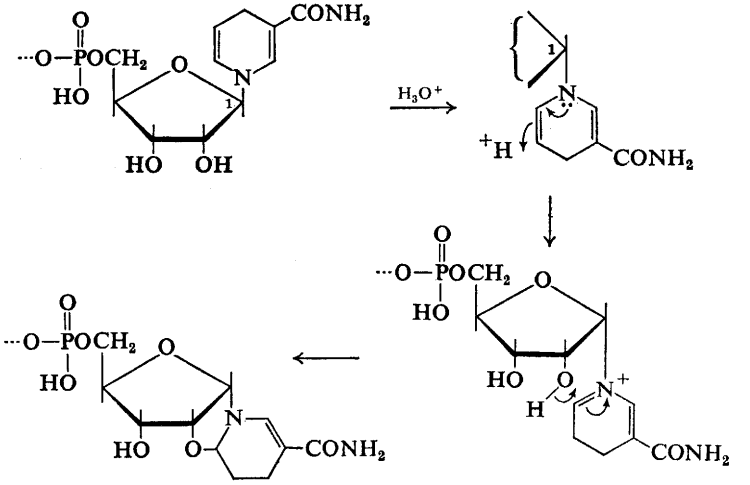
The hydrolyses of thymidine and 2′-deoxyuridine with acid also resulted in formation of α-furanosyl and α- and β-pyranosyl isomers, suggesting that the reaction involves initial protonation of O-4′ (see Scheme 127), whereas similar hydrolyses of 5-bromo-2′-deoxyuridine and 2′-deoxycytidine resulted in cleavage of the *N*-glycosyl bond only.[681] The bisulphite adducts (5,6-dihydro-6-sulphonates) of thymidine and 2′-deoxyuridine also underwent anomerization, furanose–pyranose equilibration, and hydrolysis on treatment with acid.[682] The rates of hydrolysis of guanosine, inosine, and adenosine with dilute hydrochloric acid and with sulphonic acid resins have been examined; differences in the rates of hydrolysis were ascribed to differences in the sites of protonation of the bases.[683] The same nucleosides underwent anomerization and furanose–pyranose equilibration with dilute alkali; since 2′-deoxy- and 2′-substituted-nucleosides were stable under alkaline conditions, the 2′-hydroxy-group is essential for these isomerizations, possibly participating in the cleavage of the C-1′—O-4′ bond.[684] Detailed studies have been performed on the acid- and alkali-catalysed decompositions of inosine.[685] 2′-Deoxynucleosides underwent thermolytic cleavage of the glycosidic bond on fusion, giving the corresponding base, furfuryl alcohol, and water; the rates of cleavage of purine and pyrimidine nucleosides were compared.[686]

The glycosylamine derivative (393) has been identified as a product of radiolysis of thymidine 5′-phosphate in aerated, aqueous solution.[687] 220 MHz N.m.r. spectroscopy has revealed that the product obtained on acidification of reduced nicotinamide dinucleotide is an epimerized cyclonucleotide (Scheme 128).[688] Guanine nucleosides were oxidized with peroxodisulphate under slightly basic conditions, and this procedure offers a promising way of selectively modifying polynucleotides at the guanine residues.[689] N^6-(Isopent-2-enyl)adenosine, a modified nucleoside present in tRNA, has been shown to undergo slow transformation into the fluorescent nucleosides (394) and (395).[690]

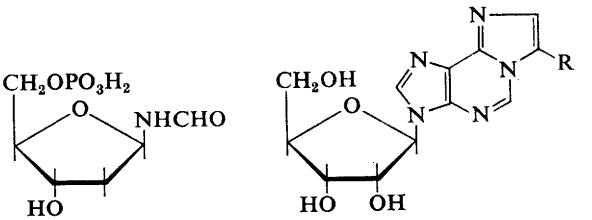
[681] J. Cadet and R. Téoule, *J. Amer. Chem. Soc.*, 1974, **96**, 6517.
[682] J. Cadet, *Tetrahedron Letters*, 1974, 867.
[683] Y. Suzuki, *Bull. Chem. Soc. Japan*, 1974, **47**, 2077.
[684] Y. Suzuki and S. Yatabe, *Bull. Chem. Soc. Japan*, 1974, **47**, 2353.
[685] Y. Suzuki, *Bull. Chem. Soc. Japan*, 1974, **47**, 2469.
[686] P. G. Olafsson and A. M. Bryan, *Arch. Biochem. Biophys.*, 1974, **165**, 46.
[687] J. Cadet, R. Ducolomb, and R. Téoule, *Compt. rend.*, 1974, **279**, *C*, 549.
[688] N. J. Oppenheimer and N. O. Kaplan, *Biochemistry*, 1974, **13**, 4675.
[689] R. C. Moschel and E. J. Behrman, *J. Org. Chem.*, 1974, **39**, 2699.
[690] G. B. Caheda, S. P. Dutta, A. Mittelman, and L. Baczynskyj, *Tetrahedron Letters*, 1974, 433.



Scheme 127



Scheme 128



(393)

(394) R = H
(395) R = Me_2COH

Physical Measurements

Chemical-shift differences between the dioxolan-methyl signals of (2,3-*O*-isopropylidene-D-ribofuranosyl)nucleosides appear to be diagnostic of the anomeric configuration; thus, for the β-anomers $\Delta\delta \geqslant 0.18$ (average 0.20) and for the α-anomers $\Delta\delta \leqslant 0.10$ (average 0.03).[691] Substitution at O-3′ or -5′ of pyrimidine nucleosides affected the chemical shift of H-6, and this effect has been used to examine the products of tritylation.[692]

Raman and Raman-difference spectroscopy have shown that the binding of methylmercury(II) cations ($MeHg^+$) occurs at N-3 of uridine and cytidine,[693] and perturbations of the vibrations of the cation and the nucleotide upon metallation of adenosine and guanosine 5′-phosphates at different sites were also determined.[694] The binding of heavy metals to nucleosides is also discussed in Chapter 18. The stabilities of charge-transfer complexes formed between adenosine, inosine, and other nucleotides and 2,2′-bipyridyl and its copper(II) complex have been determined by spectometric methods.[695]

[691] J.-L. Imbach, J.-L. Barascut, B. L. Kam, and C. Tapiero, *Tetrahedron Letters*, 1974, 129.
[692] W. Regel, E. Stengele, and H. Seliger, *Chem. Ber.*, 1974, **107**, 611.
[693] S. Mansy, T. E. Wood, J. C. Sprowles, and R. S. Tobias, *J. Amer. Chem. Soc.*, 1974, **96**, 1762.
[694] S. Mansy and R. S. Tobias, *J. Amer. Chem. Soc.*, 1974, **96**, 6874.
[695] C. F. Naumann and H. Sigel, *J. Amer. Chem. Soc.*, 1974, **96**, 2750.

Ultrasonic-absorption measurements in the range 10—300 MHz, which enable the thermodynamics of fast isomerization processes to be measured, have been applied to a study of the *syn–anti* conformational equilibria of nucleosides; this technique revealed that both *syn* and *anti* conformers of adenosine are significantly populated at ambient temperatures.[696]

Further reference to conformational studies of nucleosides by n.m.r. methods is made in Chapter 23, and other physical measurements on nucleosides are contained in Chapters 24 and 25.

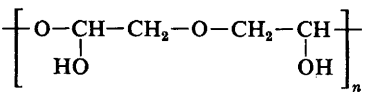
[696] P. R. Hemmes, L. Oppenheimer, and F. Jordan, *J. Amer. Chem. Soc.*, 1974, **96**, 6023.

22
Oxidation and Reduction

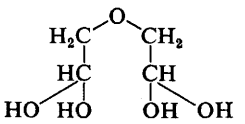
Oxidation

Periodate oxidation of a variety of organic compounds, including carbohydrates, has been reviewed.[697] Spectrophotometric and the Fleury–Lange methods used for determining the uptake of periodate and the formation of formic acid have been compared in alkaline and acidic media for oxidation of glycerol, D-glucose, and D-mannitol; it was concluded that alkaline media are not suitable for these determinations owing to over-oxidation.[698]

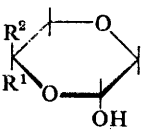
A fully automated system has been developed for hypoiodite oxidation of aldoses and substituted aldoses to the corresponding aldonic acids; determination of the glyoxylic acid and formaldehyde liberated on oxidation of the derived aldonic acid with periodate permitted 3-, 4-, and 6-*O*-substituted aldonic acids to be distinguished.[699] The dialdehyde obtained by periodate oxidation of 1,5-anhydropentitols and 1,4-anhydrotetritols has been shown to exist in a variety of forms.[700] In syrupy form the dialdehyde is largely polymeric (396),



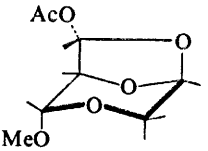
(396)



(397)



(398) R^1 = OH; R^2 = H
(399) R^2 = H; R^1 = OH



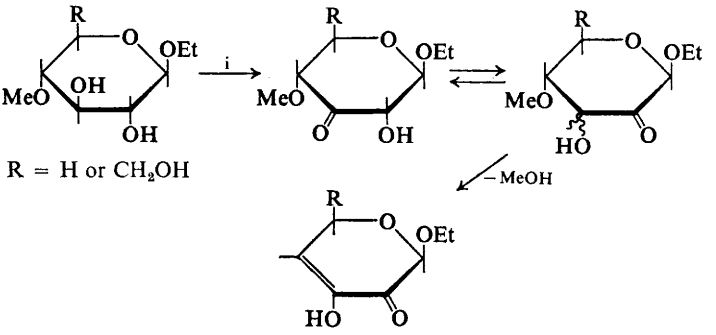
(400)

whereas a hydrated acyclic form (397) and *cis*- and *trans*-1,4-dioxan-2,6-diols (398) and (399), respectively, are present in solution, the proportions of the three forms at equilibrium depending on the solvent and the temperature. Unimolar

[697] A. J. Fatiadi, *Synthesis*, 1974, 229.
[698] K. Babor, V. Kalac, and K. Tihlarik, *Chem. Zvesti*, 1973, **27**, 676 (*Chem. Abs.*, 1974, **80**, 96 233d).
[699] A. M. Y. Ko and P. J. Somers, *Carbohydrate Res.*, 1974, **34**, 57.
[700] H. R. Greenberg and A. S. Perlin, *Carbohydrate Res.*, 1974, **35**, 195.

oxidation of methyl β-L-arabinopyranoside with periodic acid in DMSO afforded, after acetylation, a single product identified as (400) by ^{1}H n.m.r. spectroscopy.[701]

The efficiency of catalysts in formation of carboxylic acids from air oxidation of di-*O*-isopropylidene derivatives of L-sorbofuranose, D-fructopyranose, and D-galactopyranose has been studied.[702] Investigations of the degradations of ethyl 4-*O*-methyl-β-D-gluco- and -xylo-pyranosides (as models for cellulose) with oxygen–alkali have indicated that the main pathway involves elimination of the substituent at C-4 following oxidation of a secondary hydroxy-group to a keto-group (Scheme 129).[703]



R = H or CH_2OH

Reagent: i, O_2–NaOH–H_2O

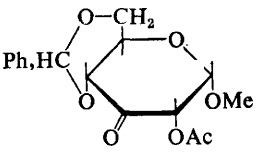
Scheme 129

Free radicals have been detected by e.s.r. spectroscopy during oxidation of carbohydrates by cobalt(III) and cerium(IV) reagents in aqueous solution.[704] Oxidation of inositol by vanadium(V) in acidic media has been shown to be first order in vanadium(V) and inositol, and to require two equivalents of vanadium(V) per mol of inositol to produce 1 mol of inosose.[705] The co-ordination of copper(II) in *NN'*-ethylenebis(trifluoroacetylacetoiminato)copper(II) has been compared with that in D-galactose oxidase (E.C. 1.1.3.9), which catalyses the conversion of D-galactose into the corresponding 6-aldehyde with reduction of molecular oxygen to hydrogen peroxide.[706]

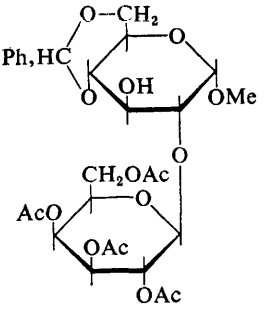
Oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with acetic anhydride-DMSO occurred preferentially at the 3-hydroxy-group to give methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-*ribo*-hexopyranosid-3-ulose (401).[707] Similar treatment of the disaccharide derivative (402) yielded only the corresponding 3-acetate, whereas oxidation with phosphorus pentoxide–DMSO furnished the required glycosulose.[708]

In continuing his studies on the oxidation of aldoses with silver carbonate on Celite (the Fetizon reagent), Morgenlie has found that prolonged treatment of

[701] J. Gelas, D. Horton, and J. D. Wander, *J. Org. Chem.*, 1974, **39**, 1946.
[702] Z. Csuros, J. Petro, E. Fogassy, and A. Lengyel, *Period. Polytech., Chem. Eng.*, 1974, **18**, 167 (*Chem. Abs.*, 1974, **81**, 105 828b).
[703] H. Kolmodin, *Carbohydrate Res.*, 1974, **34**, 227.
[704] V. I. Kurlyankina, V. N. Shadrin, E. N. Kazbekov, V. A. Molotkov, and M. K. Bukina, *Zhur. obshchei Khim.*, 1974, **44**, 1593.
[705] A. Kumar and R. N. Mehrotra, *Internat. J. Chem. Kinetics*, 1974, **6**, 15.
[706] R. S. Giordano and R. D. Bereman, *J. Amer. Chem. Soc.*, 1974, **96**, 1019.
[707] J. Defaye and A. Gadelle, *Carbohydrate Res.*, 1974, **35**, 264.
[708] Z. Pawlak, A. Temeriusz, and A. Mioduszewska, *Roczniki Chem.*, 1973, **47**, 2373.



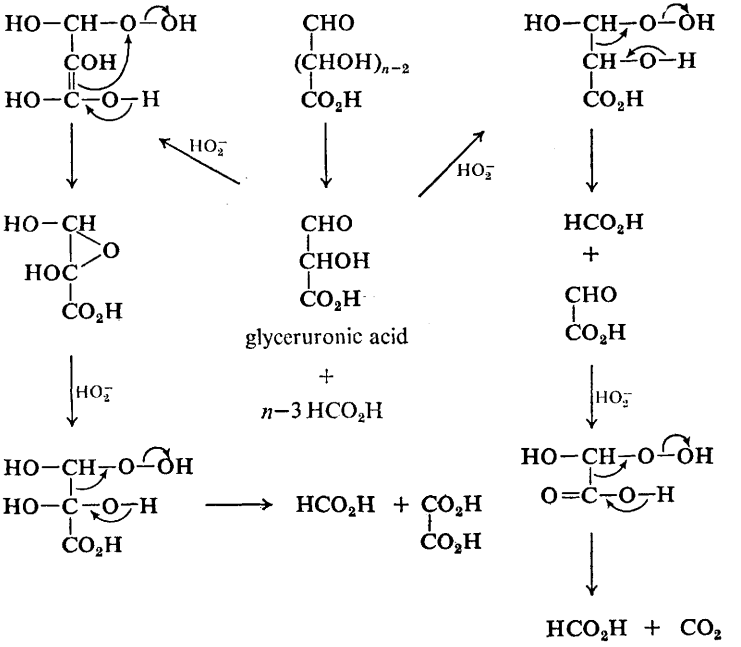
(401)



(402)

pentoses led to extensive degradation of the initial oxidation products (tetrose formyl esters and pentonolactones) to two- and three-carbon fragments,[709] and that glycolaldehyde and glyceraldehyde were oxidized in methanol to the methyl esters of the corresponding carboxylic acids.[710] The Fetizon reagent has also been used to oxidize the anomeric hydroxy-group of partially protected aldoses, forming the corresponding lactones, although unsaturated products were also obtained [498] (see p. 116).

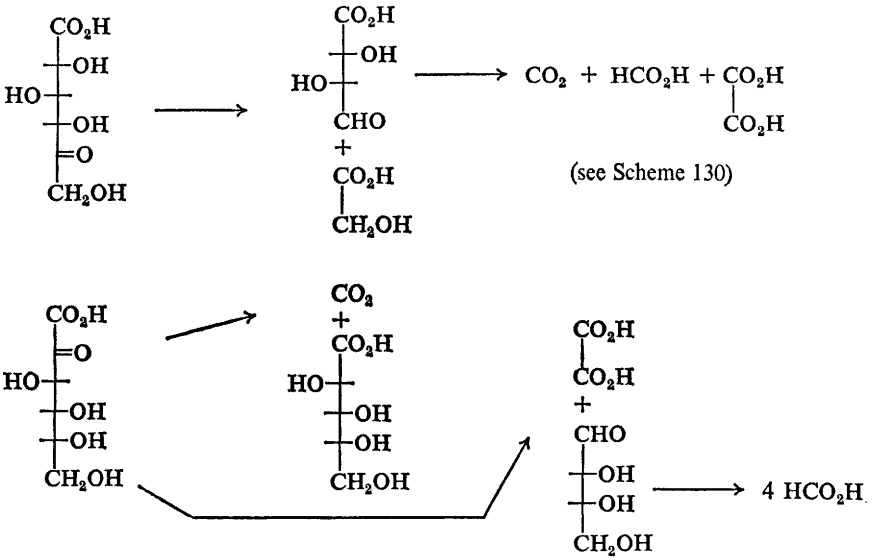
Studies by Isbell's group on the degradation of carbohydrates with alkaline hydrogen peroxide have been extended to include alduronic acids and glyculosonic



Scheme 130

[709] S. Morgenlie, *Acta Chem. Scand.*, 1973, **27**, 2607.
[710] S. Morgenlie, *Acta Chem. Scand.*, 1973, **27**, 3009.

acids.[711] Alduronic acids were degraded to formic acid and carbon dioxide by the pathway previously proposed (see Vol. 7, p. 10), although oxalic acid was formed by a second pathway from the intermediate glyceruronic acid (see Scheme 130). The main pathways followed in the degradations of representative hex-2- and -5-ulosonic acids with alkaline peroxide are indicated in Scheme 131.

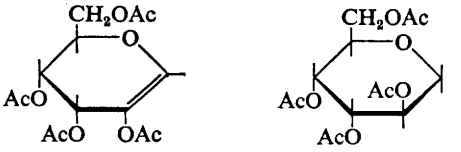


Scheme 131

Of possible interest to carbohydrate chemists is the observation that secondary hydroxy-groups of steroids can be oxidized in the presence of primary hydroxy-groups using pyridine–chlorine complexes in chloroform.[712]

Reduction

Hydrogenation of D-glucose in the presence of a composite catalyst of nickel, aluminium, and palladium gave D-glucitol in high yield.[713] It is notable that hydrogenation of the 2-acetoxyglycal (403) over palladium black afforded 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-altritol (404), although the isomeric D-allitol derivative might be expected from steric considerations.[437]



(403) (404)

[711] H. S. Isbell, H. L. Frush, and Z. Orhanovic, *Carbohydrate Res.*, 1974, **36**, 283.
[712] J. Wicha and A. Zarecki, *Tetrahedron Letters*, 1974, 3059.
[713] N. K. Nadirov, Sh. Kh. Khandodzhaev, and A. M. Ashirov, *Khim. Khim. Tekhnol. (Alma Ata)*, 1971, No. 2, 80.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

A number of reviews on spectroscopic and conformational aspects of carbohydrates have appeared during the past year. A paper in the Van't Hoff–Le Bel Commemorative Issue of *Tetrahedron* contains a general discussion on the conformational consequences of replacing a methylene group by an oxygen atom in cyclic compounds, and has obvious implications for carbohydrate chemistry.[714] A general review (in French) of the applications of n.m.r. spectroscopy to carbohydrates has appeared,[715] while more specialized reviews have discussed the following: solutions to the hidden-resonance problem in ^{1}H n.m.r. spectroscopy caused by overlapping signals;[716] the relation between vicinal $J_{^{13}C,^1H}$ coupling constants and torsion angles in nucleosides and glycosides;[717] the conformational equilibria of acylated aldopentopyranoses and the preferred conformations of acyclic derivatives;[718, 719] the conformations of carbohydrates in the solid state and whether or not extrapolation of the data to carbohydrates in solution is meaningful;[720] ^{13}C and ^{1}H n.m.r. spectroscopy of ketohexoses in various solvents;[721] and the conformations of nucleosides about the *N*-glycosyl and phosphate bonds.[582]

Lemieux and Koto have discussed recent evidence concerning the factors responsible for the anomeric effect and the *exo*-anomeric effect (*i.e.* the preferred orientation of the O-1–aglycone bond relative to the sugar ring),[722] and a quantum-mechanical analysis of the anomeric (gauche) effect has been presented using Altona's model based on the interaction of one of the lone pairs of electrons on the ring-oxygen atom with adjacent antibonding orbitals.[723] Thus, the equilibrium conformation was considered to reflect a compromise between electronic stabilization and core repulsion, whose contribution can be included using the Jahn–Teller second-order effect. A study of the *cis–trans* equilibria of 2-mono- and 2,2-di-substituted 1,3-dioxan derivatives as a function of solvent has provided additional information on the anomeric and reverse anomeric effects.[724]

[714] J. Dale, *Tetrahedron*, 1974, **30**, 1683.
[715] M. Vincendon, *Bull. Soc. chim. France*, 1973, 3500.
[716] L. D. Hall, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 11.
[717] R. U. Lemieux, *Ann. New York Acad. Sci.*, 1973, **222**, 915.
[718] P. L. Durette, D. Horton, and J. D. Wander, in 'Carbohydrates in Solution', Advances in Chemistry Series No. 117, American Chemical Society, New York, 1973, p. 147.
[719] D. Horton, P. L. Durette, and J. D. Wander, *Ann. New York Acad. Sci.*, 1973, **222**, 884.
[720] G. A. Jeffrey, in ref. 718, p. 177.
[721] A. S. Perlin, P. C. M. Herve du Penhoat, and H. S. Isbell, in ref. 718, p. 39.
[722] R. U. Lemieux and S. Koto, *Tetrahedron*, 1974, **30**, 1933.
[723] R. Ponec and V. Chvalovský, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2613.
[724] W. F. Bailey and E. L. Eliel, *J. Amer. Chem. Soc.*, 1974, **96**, 1798.

Such studies with ethynyl and substituted-phenyl derivatives indicated that the anomeric effect is not entirely caused by dipole interactions but, at least in part, by double bond–no bond resonance, as originally proposed by Altona. Tenuous evidence for the operation of the reverse anomeric effect was obtained for 2-chloromethyl- and 2-bromomethyl-2,4-dimethyl-1,3-dioxans.

^{15}N N.m.r. spectra have been recorded for 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-[^{15}N]phthalimido-α-D-galactopyranose and 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-[^{15}N]phthalimido-α-D-glucofuranose, both at natural abundance and on isotopically enriched samples; the difficulties associated with the technique, which shows promise in structural analysis, were discussed.[725] ^{19}F N.m.r. spectroscopy has been used to study the binding of 2-deoxy-2-trifluoroacetamido-α- and -β-D-glucopyranoses to concanavalin A.[726]

The relative merits and accuracy of pulse Fourier-transform and continuous-wave spectroscopic methods have been delineated in a paper dealing with conformational aspects of acylated 1,1-bis(acylamido)-1-deoxypentitols (see also p. 170); one advantage of the former method is that mathematical convolution techniques may be applied to improve the quality of the spectra, by enhancement of either resolution or sensitivity.[727]

The use of CIDNP techniques to study the formation of radicals is referred to in Chapter 2.

Pyranoid Systems

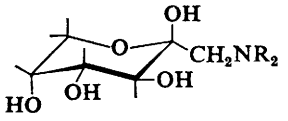
The potential and free energies of methyl aldo-hexo- and -pento-pyranosides in their $^4C_1$ and $^1C_4$ conformations have been calculated using a MO-LCAO method.[728] Considerable relief of strain is obtained when tilts of 4.5° and 3° are given to axial hydroxymethyl- and hydroxy-groups, respectively, that are involved in the Hassel–Ottar effect. The calculated free-energy values agreed well with experimental values, after adding 0.8 kcal mol^{-1} for the anomeric effect of the methoxy-group, and it was predicted that all the methyl D-aldohexopyranosides, except methyl α- and β-D-idopyranosides, should favour the $^4C_1$ conformation. The calculated energies also predict that only methyl α- and β-D-xylopyranosides and methyl α-D-ribopyranoside of the methyl aldopentopyranosides should favour the $^4C_1$ conformation, whereas others should contain significant proportions of both $^4C_1$ and $^1C_4$ conformations at equilibrium. This approach has been extended to a study of the conformational equilibria of aldopentopyranose tetra-acetates to afford more information about the stereochemistry of the pyranoid rings.[729] *Ab initio* MO calculations performed on methoxymethanol, a structural analogue of the hemiacetal and acetal moieties in aldopyranoses and methyl aldopyranosides, confirmed the favoured conformations already deduced from calculations made on dihydroxymethane.[730] It was predicted that the O—CH_3 bond in methyl aldopyranosides is lengthened relative to that in methanol, a prediction adequately supported by crystal-structure data.

[725] B. Coxon, *Carbohydrate Res.*, 1974, **35**, C1.
[726] G. M. Alter and J. A. Magnuson, *Biochemistry*, 1974, **13**, 4058.
[727] B. Coxon, R. S. Tipson, M. Alexander, and J. O. Deferrari, *Carbohydrate Res.*, 1974, **35**, 15.
[728] K. S. Vijayalakshmi, N. Yathindra, and V. S. R. Rao, *Carbohydrate Res.*, 1973, **31**, 173.
[729] V. S. R. Rao and K. S. Vijayalakshmi, *Carbohydrate Res.*, 1974, **33**, 363.
[730] G. A. Jeffrey, J. A. Pople, and L. Radom, *Carbohydrate Res.*, 1974, **38**, 81.

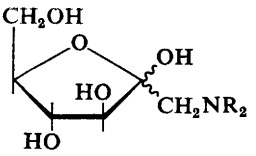
2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl chloride has been shown to crystallize in the $^4C_1$ conformation, although it exists mostly (80%) in the $^1C_4$ conformation in solution,[731] whereas (2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)imadazole crystallizes in the $^1C_4$ conformation, suggesting that the reverse anomeric effect is operating.[732] The conformations of a series of trimethylsilylated sugars have been examined by high resolution (220 and 300 MHz) ^{1}H n.m.r. spectroscopy. Thus, it was concluded that TMS-α- and -β-D-fructopyranoses occur in the $^2C_5$ conformation, and that the ^4E and $^4T_5$ conformations are assumed by the TMS-D-fructofuranose rings in such oligosaccharides as sucrose, 1-kestose, and melezitose.[733] All the pyranoid rings of TMS derivatives of β-D-altro-, β-D-allo-, and α- and β-D-talo-pyranose and of a number of 6-deoxyaldohexopyranoses occur in the $^4C_1$ or $^1C_4$ conformation, and the TMS group substituted at O-6 did not appear to affect the conformation of the ring.[733a] Per-trimethylsilylated 2-acetamido-2-deoxy derivatives of α- and β-D-galacto-, -gluco-, and -manno-pyranose were each shown to exist in the $^4C_1$ conformation,[734] and examination of the unmodified acetamido-sugars in DMSO (a hydrogen-bond proton acceptor) and 1,1,1,3,3,3-hexafluoropropan-2-ol (a hydrogen-bond proton donor) also indicated the $^4C_1$ conformation of the pyranoid rings.[735] The effect of the 4-hydroxy-group on the conformational preferences of the 6-hydroxymethyl-group in water, DMSO, and 1,2-dichloroethane, *etc.*, has been assessed by a comparison of 1,5-anhydro-2,3,4-trideoxy-D-*glycero*-hexitol and 1,5-anhydro-2,3-dideoxy-D-*erythro*- and -*threo*-hexitols and methyl ethers derived therefrom; the effect of the ring-oxygen atom was also studied by comparison with the diastereoisomeric 2-hydroxymethylcyclohexanols.[736]

The conformational equilibria ($^0H_1 \leftrightarrows {}^1H_0$) of twenty-six diastereoisomeric methyl 3,4-dideoxy-DL-glyc-3-enopyranosides have been determined on the basis of $J_{1,2}$ values.[737] The anomeric effect, 1,3-*syn*-axial repulsion, and the preference for the substituent at C-6 to adopt a pseudo-equatorial orientation contribute to the position of the conformational equilibrium, whereas the contribution of the allylic effect appears to be restricted to 2-*O*-acetyl derivatives. A related study of the conformational equilibria ($^4H_5 \leftrightarrows {}^5H_4$) of acetylated glycals has utilized the observation that $^4J_{2,4}$ is large when H-4 is equatorial, but is not observed when H-4 is axial.[738] Both ^{1}H and ^{13}C n.m.r. spectroscopy have been used to study the conformation of the six-membered ring of D(and L)-inositol phenylosotriazole,[739] and 220 MHz ^{1}H n.m.r. spectroscopy has revealed that Amadori rearrangement of D-glucose produces an equilibrium mixture of the β-pyranosyl- and αβ-furanosyl-amines, (405) and (406), respectively.[740]

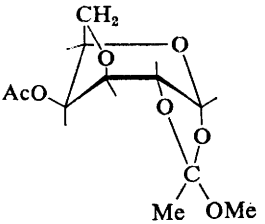
[731] G. Kothe, P. Luger, and H. Paulsen, *Carbohydrate Res.*, 1974, **37**, 283.
[732] P. Luger, G. Kothe, and H. Paulsen, *Chem. Ber.*, 1974, **107**, 2626.
[733] D. G. Streefkerk, M. J. A. De Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **33**, 249.
[733a] D. G. Streefkerk, M. J. A. De Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **38**, 47.
[734] D. G. Streefkerk, M. J. A. De Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **33**, 339.
[735] T. J. Schamper, *Carbohydrate Res.*, 1974, **36**, 233.
[736] R. U. Lemieux and J. T. Brewer, in ref. 718, p. 121.
[737] O. Achmatowicz, A. Banaszek, M. Chmielewski, A. Zamojski, and W. Lobodzinski, *Carbohydrate Res.*, 1974, **36**, 13.
[738] A. A. Chalmers and R. H. Hall, *J.C.S. Perkin II*, 1974, 728.
[739] A. J. Fatiadi, *Carbohydrate Res.*, 1974, **35**, 280.
[740] S. B. Tjan and G. A. M. van der Ouweland, *Tetrahedron*, 1974, **30**, 2891.



(405)



(406)



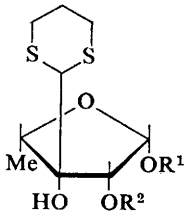
(407)

^{1}H N.m.r. spectroscopy has indicated that the orthoester (407) adopts a $B_{1,4}$ rather than a $^1C_4$ conformation,[95] and studies of the base-catalysed equilibration and conformations of some methyl 2,3- and 3,4-anhydro-6-deoxy-β-D-hexopyranosides have been mentioned in Chapter 4.

The spectra (in D_2O) of partially methylated D-glucopyranosides obtained from poly(D-glucoses) showed signals at δ 3.39—3.41 for 6-substituted methoxy-groups, at *ca.* δ 3.61 for 3-substituted methoxy-groups, at δ 3.54—3.56 for 4-substituted methoxy-groups, and at δ 3.48 and 3.59 for 2-substituted methoxy-groups in α- and β-glycosides, respectively; this information was used to analyse the structures of two glucans (coriolan and glycogen), and gave results in agreement with those previously published.[741]

Furanoid Systems

A study of the conformations of various derivatives of 1,2-*O*-isopropylidene-α-D-glucofuranose has shown that, despite the presence of the fused acetal ring, the furanoid ring still has considerable mobility.[742] Refined ^{1}H n.m.r. spectroscopic data for pentakis-*O*-trimethylsilyl-β-D-galactofuranose in [$^2H_6$]acetone has indicated that the ^4E conformation makes a major contribution to the actual structure of the furanoid ring, and that the C-5—C-6 side-chain assumes a *trans*-coplanar arrangement.[743] *X*-Ray crystallographic analysis has shown that the 1,2-*O*-isopropylidenestreptose derivative (409) crystallizes with the furanoid



(408) $R^1 = R^2 = H$
(409) $R^1, R^2 = CMe_2$

[741] T. Terui, T. Yadomae, H. Yamada, O. Hayashi, and T. Miyazaki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2476.
[742] A. A. Akhrem, G. V. Zaitseva, A. S. Friedman, and I. A. Mikhailopulo, *Spectroscopy Letters*, 1974, **7**, 1 (*Chem. Abs.*, 1974, **81**, 4154y).
[743] D. G. Streefkerk, M. J. A. De Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **33**, 350.

ring in the $^3T_4$ conformation, whereas that of the parent compound (408) assumes the $^2T_3$ conformation.[744]

As in previous years, most of the reports in this section are concerned with the applications of theoretical calculations, n.m.r. spectroscopy, and *X*-ray crystallography to nucleosides and their derivatives. Calculations of the conformations of the furanoid ring of nucleosides by the atom–atom potential method have predicted that the $^3T_2$ and $^2T_3$ conformations should be preferred.[745] MO calculations made on β-D-arabinofuranosylnucleosides have predicted an *anti*-arrangement about the glycosyl bond for pyrimidine nucleosides adopting either the ^2E or ^3E conformation of the furanoid ring, provided that there is no intramolecular hydrogen bonding between O-5′ and O-2; the *syn* arrangement is equally probable for the ^2E conformation if such a hydrogen bond is present.[746] The calculations also indicated that a *syn* arrangement is preferred for purine nucleosides adopting the ^2E and ^3E conformations of the furanoid ring and with hydrogen bonding between either O-5′ and N-3 or O-5′ and O-2′, whereas an *anti* arrangement is more likely in the absence of such hydrogen bonding. In all cases, the *gauche–gauche* conformation about the C-4′—C-5′ bond is preferred. Available *X*-ray and solution data are in excellent agreement with these conclusions. Similar calculations made on nucleoside 2′,3′- and 3′,5′-cyclic phosphates were also found to be in good agreement with experimental data,[747] and there are clear indications that the relative orientations of the sugar and base rings of nucleoside 3′,5′-cyclic phosphates depend on the particular base involved.[748] The furanoid ring of adenosine 3′,5′-cyclic phosphate was predicted by extended Hückel theory to prefer the $^3T_4$ conformation, with the phosphate ring in a chair conformation.[749]

MO (INDO) calculations made on nucleosides have predicted long-range couplings between H-1′ and the protons of the base moiety and between the protons on the furanoid ring.[750] The calculations indicated that long-range coupling is negligible for the protons of purine bases, whereas such coupling is very sensitive to the sugar–base torsion angle for the protons of pyrimidine bases; *e.g.*, the magnitude of $^5J_{1',5}$ for uridine is compatible with an *anti* arrangement about the glycosyl bond. However, long-range coupling between the protons on the furanoid ring showed little variation with puckering of the ring.

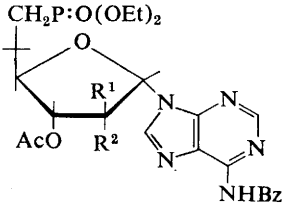
One approach used to determine the anomeric configuration of nucleosides has been mentioned in Chapter 21. Another approach, related to that already used with acetylated pyrimidine nucleosides, has been based on the observation that the 2′-acetoxy-group signals of 1′,2′-*cis*-furanosylpurine nucleosides occur 0.11—0.34 p.p.m. upfield from the highest signals from the corresponding *trans*-nucleoside.[751] Thus, all the acetoxy-group signals for acetylated β-D-ribofuranosyl-, α-D-arabinofuranosyl-, and β-D-xylofuranosyl-purine nucleosides are

[744] W. Depmeier, O. Jarchow, P. Stadler, V. Sinnwell, and H. Paulsen, *Carbohydrate Res.*, 1974, **34**, 219.
[745] A. A. Lugovskoi and A. I. Kitaigorodskii, 'Konformatsionnye Izmen. Biopolim. Rastrorakh. Vses. Soveshch. [Doklady]', 1971 (pub. 1973), p. 86.
[746] A Saran, B. Pullman, and D. Perahia, *Biochem. Biophys. Acta*, 1974, **349**, 189.
[747] A. Saran, H. Berthod, and B. Pullman, *Biochim. Biophys. Acta*, 1973, **331**, 154.
[748] N. Yathindra and M. Sundaralingam, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 119.
[749] J. N. Lespinasse and D. Vasilescu, *Biopolymers*, 1974, **13**, 63.
[750] C. Giessner-Prettre and B. Pullman, *Internat. J. Quantum Chem., Symp.*, 1973, **7**, 295.
[751] J. A. Montgomery, *Carbohydrate Res.*, 1974, **33**, 184.

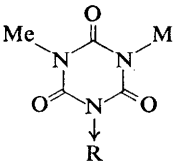
found *downfield* from 2.05 p.p.m., whereas one of the acetoxy-group signals for acetylated β-D-arabinofuranosyl-, α-D-xylofuranosyl-, and α-D-ribofuranosyl-purine nucleosides appears upfield from 1.95 p.p.m. A nucleoside derivative previously assigned the α-D-*ribo*-configuration (410) was established to have the α-D-*arabino*-configuration (411) using this method, since both acetoxy-group signals appeared *downfield* from 2.05 p.p.m. The preferred conformational features of common ribo- and deoxyribo-nucleoside 5′-phosphates, revealed by 220 MHz ^{1}H n.m.r. spectroscopy, were a *gauche–gauche* arrangement about the C-4′—C-5′ bond, an *anti* relationship between the base and sugar rings, and a $^2T_3$ rather than a $^3T_2$ conformation of the furanoid ring.[752] The 100 MHz spectra of cytidine, 1-β-D-arabinofuranosylcytosine, and *O*-methyl ethers thereof have been measured and analysed by computer simulation; the rotamer states of various portions of the molecules, including those of the *O*-methyl groups, were determined.[753] The molecular structure of 9-β-D-arabinofuranosyladenine has been determined, and the following conformational features were revealed: a ^3E conformation of the furanoid ring, a *gauche–trans* arrangement about the C-4′—C-5′ bond, and an *anti* arrangement about the glycosyl bond.[754] N.m.r. spectroscopic examination of 2′-deoxy-2′-fluoro-cytidine and -uridine and derivatives thereof has shown that the furanoid ring in each adopts a $^3T_4$ conformation, with a small contribution from the ^2E conformation,[755] and related studies with 2-deoxythymidine, 3′- and 5′-phosphates thereof, and the derived 3′,5′-dinucleotide have revealed that substitution at O-3′ and O-5′ produces only slight changes of the conformation.[756] Conformational features of ribovirin and its 5′-phosphate have been studied by 220 MHz ^{1}H n.m.r. spectroscopy,[757] which also demonstrated the existence of virtual coupling between H-1′ and H-3′ in 2′-*O*-benzyladenosine.[758] The effect of 2′-*O*-methyl groups on the conformations of pyrimidine nucleosides has been reinvestigated by pseudo-rotational analysis of the furanoid ring; *O*-methylation and changes of temperature did not appear to alter the conformational parameters of the furanoid ring, but did affect the equilibrium between ^2E and ^3E conformations.[758a]

The barrier to internal rotation about the glycosyl bond in nucleosides has been estimated using derivatives of the symmetrical bases (412) and (413), which removed the need to distinguish between *syn* and *anti* rotamers; $\Delta G^{\ddagger}$ values of *ca.* 17 and 13 kcal mol^{-1} were determined for (412) and (413), respectively, and these values may be related to the angles subtended by the carbonyl groups of the heterocyclic rings.[759] For a series of structurally related pyrimidine nucleosides in aqueous solution, it was noted that the difference in chemical shift between the H-5′ protons is proportional to the sum of the observed $J_{4',5'}$ values.[760]

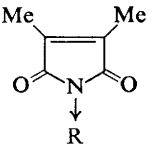
[752] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1974, **13**, 4417.
[753] M. Remin and D. Shugar, *J. Amer. Chem. Soc.*, 1973, **95**, 8146.
[754] G. Bunick and D. Voet, *Acta Cryst.*, 1974, **B30**, 1651.
[755] M. Blandin, Tran Dinh Son, J. C. Catlin, and W. Guschlbauer, *Biochim. Biophys. Acta*, 1974, **361**, 249.
[756] D. J. Wood, F. E. Hruska, and K. K. Ogilvie, *Canad. J. Chem.*, 1974, **52**, 3353.
[757] P. Dea, M. P. Schweizer, and G. P. Kreishman, *Biochemistry*, 1974, **13**, 1862.
[758] A. D. Broom and L. F. Christensen, *J. Org. Chem.*, 1974, **39**, 2660.
[758a] D. B. Davies, *J.C.S. Perkin II*, 1974, 915.
[759] H. von Voithenberg, A. Skrzelewski, J. C. Jochims, and W. Pfleiderer, *Tetrahedron Letters*, 1974, 4063.
[760] F. E. Hruska, D. J. Wood, T. N. McCaig, A. A. Smith, and A. Holý, *Canad. J. Chem.*, 1974, **52**, 497.



(410) R^1 = H; R^2 = OAc
(411) R^1 = OAc; R^2 = H



(412)



(413)

R = 2,3,4,6-tetra-*O*-acetyl(benzoyl)-*β*-D-glucopyranosyl

Adenosine 5′-phosphate[761] and *β*-pseudouridine[761a] have been examined by nuclear-Overhauser techniques; in the latter case, the *syn–anti* equilibrium was shown to be independent of pH over the pH range 7.1—11.6, and CNDO calculations suggested that the *syn* arrangement is stabilized by hydrogen bonding between the carbonyl group at C-4 and the hydroxy-group at C-5′.

Di-, Oligo-, and Poly-saccharides

High-resolution ^1H n.m.r. spectroscopy has permitted the ring protons of a number of acetylated mono-, di-, and tri-*O*-trityl derivatives of sucrose to be assigned, and the anisotropic shielding effects of the trityl group were discussed.[762] The ^1H n.m.r. spectra of a number of unmodified D-gluco-oligosaccharides and D-glucans in D_2O have been studied with respect to the chemical shifts and coupling constants of the anomeric protons.[763] The anomeric-proton signals of a series of oligosaccharides have been analysed by measuring the spectra in mixtures of [^{2}H]chloroform and [$^2H_4$]methanol, sometimes with D_2O added; the signals for the anomeric protons appeared at δ 4.20—5.50, and the signals for the hydroxylic protons were removed to lower field by the addition of trifluoroacetic acid.[764] Saturated and unsaturated oligo(D-galactopyranuronic acids), obtained by enzymic degradation of purified pectic acid, and fully and partly esterified methyl esters thereof have been examined by high-resolution ^1H n.m.r. spectroscopy in D_2O.[765] The δ values and coupling constants confirmed that all the saturated D-galacturonic acid residues adopt the $^4C_1$ conformation and are α-(1 → 4)-linked. The double bond in the unsaturated oligo(D-galactopyranuronic acids) was shown to be located between C-4 and C-5 of the non-reducing, terminal unit, which occurs in the $^2H_1$ conformation. Structure–taste relationships have been performed on none (1 → 2)-linked glycosyl-D-glucoses (*e.g.* kojibiose and sophorose).[766] Although nine of the disaccharides scored a sweetness value approaching that of sucrose, each of the four sweetest members had a $^4C_1$(D) or (L) conformation and each had an axial O-1′ atom *cis*

[761] Tran Dinh Son, C. Chachaty, and M. Gueron, *J. Physiol. (Paris), Colloq.*, 1973, 57 (*Chem. Abs.*, 1974, **81**, 91 859y).
[761a] R. K. Nanda, R. Tewari, G. Govil, and I. C. P. Smith, *Canad. J. Chem.*, 1974, **52**, 371.
[762] T. Otake, *Bull. Chem. Soc. Japan*, 1974, **47**, 1938.
[763] T. Usui, M. Yokoyama, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *Carbohydrate Res.*, 1974, **33**, 105.
[764] K. Miyahara and T. Kawasaki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1407.
[765] S. B. Tjan, A. G. J. Voragen, and W. Pilnik, *Carbohydrate Res.*, 1974, **34**, 15.
[766] W. E. Dick, jun., J. E. Hodge, and G. E. Inglett, *Carbohydrate Res.*, 1974, **36**, 319.

to O-2′, *viz.* 2-*O*-α-D-xylopyranosyl-, 2-*O*-β-L-arabinopyranosyl-, 2-*O*-α-D-glucopyranosyl-, and 2-*O*-α-D-galactopyranosyl-D-glucose.

Acyclic Derivatives

A study of the conformations of acylated 1,1-bis(acylamido)-1-deoxypentitols by ^{1}H n.m.r. spectroscopy showed that the *arabino*- and *lyxo*-isomers adopt planar, zigzag conformations, whereas the *ribo*- and *xylo*-isomers favour sickle conformations with C-1 and C-5, respectively, displaced from the plane formed by the other carbon atoms.[727] The coupling constants pointed to an unambiguous conformation only in the case of the *xylo*-isomer, although the presence of unfavourable 1,3-interactions in the other cases allowed alternative conformations to be excluded. Related studies on D-aldopentose diethyl and diphenyl dithioacetals in solution have also indicated that a quantitative description of the conformational behaviour of acyclic carbohydrates in terms of simple substituent–interaction parameters is unlikely to be achieved.[206]

Complex formation between alditols and praseodymium nitrate has been studied by ^{1}H n.m.r. spectroscopy [767] (see also Chapter 27).

Lanthanide Shift Reagents

Addition of lanthanide ions to *epi*-inositol and 1,6-anhydro-β-D-allopyranose in aqueous solution caused shifts in their ^{1}H n.m.r. spectra that were attributed to contact and pseudo-contact interactions.[767a] Complexing in these compounds occurs at an *ax.*, *eq.*, *ax.* sequence of three oxygen atoms, and the contact interaction was demonstrated to be greatest when the bonds connecting the proton and the cation form a planar, zigzag arrangement. A chiral shift reagent, tris[(3-heptafluorobutyl)camphorato]europium(III), has been used to determine the optical purity of alcohols, but it should be used with the greatest care in determining absolute chiralities.[768] N.m.r. studies with Eu(dpm)$_3$ have demonstrated that it did not produce any changes in the conformations of phenyl 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-gluco- and -galacto-pyranosides,[769] and the molecular associations of Eu(dpm)$_3$ and Eu(fod)$_3$ with carbohydrate derivatives containing acetal, hydroxy-, methoxy-, and acetoxy-groups have been studied.[770] It appeared that Eu(fod)$_3$ reagents gave the best results when the principal binding site was a hydroxy-group, and non-linear plots [sugar concentration *versus* induced chemical-shift change] were obtained with the shift reagents in chloroform solutions.

Two papers have described studies on adenosine 3′,5′-cyclic phosphate using lanthanide ions as shift [771, 772] and relaxation [772] probes.

[767] T. Spoormaker, A. P. G. Kieboom, A. Sinnema, J. M. van der Toorn, and H. van Bekkum, *Tetrahedron Letters*, 1974, 3713.
[767a] S. J. Angyal, D. Greeves, and V. A. Pickles, *J.C.S. Chem. Comm.*, 1974, 589.
[768] G. R. Sullivan, D. Ciavarella, and H. S. Mosher, *J. Org. Chem.*, 1974, **39**, 2411.
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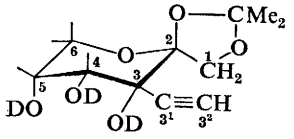
[770] A. Arduini, I. M. Armitage, L. D. Hall, and A. G. Marshall, *Carbohydrate Res.*, 1973, **31**, 255.
[771] D. K. Lavallee and A. H. Zeltmann, *J. Amer. Chem. Soc.*, 1974, **96**, 5552.
[772] C. O. Barry, D. R. Martin, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 491.

^{13}C N.M.R. Spectroscopy

The value of ^{13}C–^{1}H coupling constants has been amply demonstrated during the past year.

The preliminary results (Vol. 7, p. 183) of Pedersen's group on geminal ^{13}C–^{1}H couplings at C-1 of monosaccharide derivatives have been reported in full.[773] ^{13}C–^{1}H Couplings have been obtained from natural-abundance ^{13}C spectra of $\alpha\beta$-D-[5,6,6-^{2}H]glucose, and such couplings were also used to confirm the configuration assigned at the chain branch of the 3-*C*-ethynyl derivative (414); thus,



(414)

the C-3^{1} signal appears essentially as a doublet ($^{2}J_{^{13}C-3^{1},^{1}H-3^{2}}$ 49.2 Hz), with only weak (<3 Hz) coupling with H-4 due to the *gauche* arrangement.[774] Similar considerations have been applied to purine nucleosides, where $^{3}J_{^{13}C-8,^{1}H-1'}$ values were observed to be in the range 0—3 Hz for torsion angles of 10—20°, and 6—10 Hz for angles of 160—180°.[775] Vicinal coupling constants in oxygen-containing systems (*e.g.* ^{1}H—C—O—^{13}C and ^{1}H—O—C—^{13}C) have been used in studies of the *exo*-anomeric effect [722] and methylated inositols.[776] A $^{3}J_{^{13}C-2,^{1}HO-1}$ value of 9.2 Hz in 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose signified an *antiperiplanar* orientation of the two nuclei.[774] The ^{13}C chemical shifts of nuclei in several sugars underwent an upfield shift (0—0.1 p.p.m.) on substitution of a proton in the β-position by a deuteron.[777] This effect can be used in the assignment of signals; *e.g.*, the C-5 signals of hexoses can be recognized by using the readily accessible 6-$^{2}H_{2}$ derivatives. Various *trans*-decalin derivatives have been studied by ^{13}C n.m.r. spectroscopy with a view to determining the long-range shielding effects of hydroxy-groups.[778]

^{13}C N.m.r. spectroscopy has been used to ascertain the proportions of the various ring forms present at equilibrium in aqueous solutions of 2-hexuloses (see Table); the assignments were based on a study of configurationally related 1,5-anhydrohexitols, *etc.*[779] Comparable results have been obtained by Perlin's group, who also reported spectral data for several derivatives (glycosides, ethers, and selectively deuteriated compounds) that were utilized in analysis of the ^{13}C n.m.r. spectrum of D-psicose.[780] Although the ^{13}C chemical-shift pattern of β-D-psicopyranose was compatible with the $^{1}C_{4}$ conformation predicted for this

[773] K. Bock and C. Pedersen, *J.C.S. Perkin II*, 1974, 293.
[774] A. S. Perlin, N. Cyr, R. G. S. Ritchie, and A. Parfondry, *Carbohydrate Res.*, 1974, **37**, C1.
[775] P. Dea, G. P. Kreishman, M. P. Schweizer, J. T. Witkowski, R. Nunlist, and M. Bramwell, 'Proceedings 1st International Conference Stable Isotopes in Chemistry, Biology, and Medicine', 1973 (Pub. 1973), 84 (*Chem. Abs.*, 1974, **81**, 49 964d).
[776] D. E. Dorman, *Ann. New York Acad. Sci.*, 1973, **222**, 943.
[777] P. A. J. Gorin, *Canad. J. Chem.*, 1974, **52**, 458.
[778] S. H. Grover and J. B. Stothers, *Canad. J. Chem.*, 1974, **52**, 870.
[779] L. Que, jun., and G. R. Gray, *Biochemistry*, 1974, **13**, 146.
[780] P. C. M. Herve du Penhoat and A. S. Perlin, *Carbohydrate Res.*, 1974, **36**, 111.

Table *Equilibrium compositions (%) of solutions of the 2-hexuloses in water (figures in parentheses are taken from ref. 780; all other values are from ref. 779)*

2-Hexulose	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Fructose	0 (< 5)	72 (60)	5 (10)	23 (30)
D-Psicose	26 (25)	21 (25)	38 (40)	15 (10)
L-Sorbose	95 (100)	0	5	0
D-Tagatose	71 (90)	15 (10)	5	9

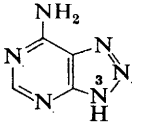
anomer, that of the α-pyranose was less readily reconciled with the predicted $^4C_1$ conformation. The anomeric configurations of four di-D-fructose dianhydrides have been assigned by ^{13}C n.m.r. spectroscopy,[781] and comparisons of the ^{13}C spectra of methyl aldo- and keto-furanosides and the corresponding pyranosides showed that the furanosides exhibit greater deshielding of ^{13}C nuclei due to the diamagnetic form of the chemical-shift value.[782]

Following work on the related D-glucose derivatives (Vol. 7, p. 183), the *O*-methyl resonances have been assigned in the ^{13}C and ^1H n.m.r. spectra of permethylated α- and β-D-galactopyranoses with the aid of specific (trideuteriomethyl)ation, heteronuclear spin-decoupling, and spectrum simulation.[783] The ^{13}C signals of α- and β-D-galactopyranose, methylated derivatives thereof, and galactitol have been assigned.[784] The work confirmed that the signal of a carbon atom bearing a hydroxy-group shows a marked downfield shift (9—10 p.p.m.) on *O*-methylation, and it was also noted that methylation of pyranoid hydroxy-groups produces an upfield shift (*ca.* 4.5 p.p.m.) of the signal of a β-carbon atom bearing an axial hydroxy-group; *e.g.*, the C-4 resonance of methyl 3-*O*-methyl-β-D-galactopyranoside appears upfield of that of methyl β-D-galactopyranoside.

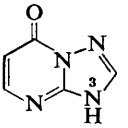
Compounds in which ^{13}C signals have been partly or completely assigned include chlorodeoxy-sugars,[308] various substituted methyl 4,6-*O*-benzylidene-D-glycopyranosides,[785] peracylated glycosyl cyanides and 1,2-*O*-cyanoethylidene-aldoses,[786] derivatives of 2-amino-2-deoxy-D-glucose, -galactose, and -mannose, and the antibiotics neomycin C and kanamycin,[787] ribostamycin (SF-733),[788] tobramycin and kanamycin B and components thereof,[789] α- and β-ribonucleosides,[790] cycloamyloses and 6-deoxy and acetylated derivatives thereof,[791] and permethylated disaccharides composed of D-glucose or D-galactose or both sugars.[792]

[781] R. W. Binkley, W. W. Binkley, and B. Wickberg, *Carbohydrate Res.*, 1974, **36**, 196.
[782] A. S. Perlin, N. Cyr, H. J. Koch, and B. Korsch, *Ann. New York Acad. Sci.*, 1973, **222**, 935.
[783] J. Haverkamp, J. P. C. M. van Dongen, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **33**, 319.
[784] W. Voelter, E. Breitmaier, E. B. Rathbone, and A. M. Stephen, *Tetrahedron*, 1973, **29**, 3845.
[785] E. Conway, R. D. Guthrie, S. D. Gero, G. Lukacs, and A.-M. Sepulchre, *J.C.S. Perkin II*, 1974, 542.
[786] B. Coxon, *Ann. New York Acad. Sci.*, 1973, **222**, 952.
[787] N. Yamaoka, T. Usui, H. Sugiyama, and S. Seto, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2196.
[788] S. Omoto, S. Inouye, M. Kojima, and T. Niida, *J. Antibiotics*, 1973, **26**, 717.
[789] K. F. Koch, J. A. Rhoades, E. W. Hagaman, and E. Wenkert, *J. Amer. Chem. Soc.*, 1974, **96**, 3300.
[790] H. Sugiyama, N. Yamaoka, B. Shimizu, Y. Ishido, and S. Seto, *Bull. Chem. Soc. Japan*, 1974, **47**, 1815.
[791] K. Takeo, K. Hirose, and T. Kuge, *Chem. Letters*, 1973, 1233.
[792] J. Haverkamp, M. J. A. De Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1974, **37**, 111.

^{13}C N.m.r. spectroscopy has been found to provide a convenient method for assigning the site of glycosylation in nucleosides containing unusual bases [*e.g.* (415) and (416)],[793] and has been used to investigate the binding of copper(II) ions to pyrimidine nucleosides and nucleotides.[794]



(415)



(416)

^{13}C N.m.r. spectroscopy has shown that the aldehydo-group of α-streptomycin is hydrated, whereas that of β-streptomycin is bound up as an intramolecular hemiacetal.[795]

Spin–Lattice Relaxation Times

A Fourier-transform method has been used to determine the spin–lattice relaxation times (T_1 values) of some of the proton resonances of monosaccharide derivatives in aqueous solution and in benzene solution.[796] The studies demonstrated that the proton spin–lattice relaxation times of pyranoses show a number of stereospecific dependences that provide a useful basis for configurational assignments. Another useful application of relaxation studies lies in the removal of unwanted, residual peaks of deuteriated solvents (*e.g.* HOD), which usually have much longer relaxation times than the protons of carbohydrates. Also of interest is the observation that the protons of β-D-galactopyranose penta-acetate have shorter relaxation times than those of the per(trideuterioacetyl)ated counterpart, indicating that there is a significant relaxation contribution from the protons of the acetyl groups.

Spin–lattice relaxation times have been measured for 3,5′-anhydro-2′,3′-*O*-isopropylideneguanosine, 2′,3′-*O*-isopropylidene-guanosine and -adenosine, and related nucleosides and nucleotides.[797] A ratio of T_1(H-8) : T_1(H-1′) > 1 was indicative of a *syn* conformation about the glycosyl bond, whereas a ratio <1 signified an *anti* conformation.

[793] P. Dea, G. R. Revankar, R. L. Tolman, R. K. Robins, and M. P. Schweizer, *J. Org. Chem.*, 1974, **39**, 3226.
[794] G. Kotowycz, *Canad. J. Chem.*, 1974, **52**, 924.
[795] K. Bock, C. Pedersen, and H. Heding, *J. Antibiotics*, 1974, **27**, 139.
[796] C. M. Preston and L. D. Hall, *Carbohydrate Res.*, 1974, **37**, 267.
[797] T. Imoto, K. Akasaka, and H. Hatano, *Chem. Letters*, 1974, 73.

24
Other Physical Methods

I.R. and Raman Spectroscopy

A book (in Russian) entitled 'Infrared Spectra and Structure of Carbohydrates' has appeared.[798]

Normal co-ordinate analysis has been used to calculate the modes of vibration for α- and β-D-glucopyranoses.[799] The calculated frequencies were found to be in good agreement with the observed i.r. and Raman values; *e.g.*, vibrations associated with the anomeric C—H bonds were predicted to occur at 845 and 901 cm^{-1} for the α- and β-anomers, respectively, whereas the values previously observed and empirically assigned occur at 840 and 898 cm^{-1}, respectively.

The i.r. spectra of acetylated pyranoses in carbon tetrachloride solution have been examined, and the relations between the configurations and the number and the positions of the absorptions were discussed.[800] Morita has similarly examined methyl, ethyl, and isopropyl glycosides, and has assigned the absorption appearing at 2865—2875 cm^{-1} in the β-anomers to the stretching of a C-1—$H_{ax.}$ bond.[801] The use of this absorption for assigning the anomeric configuration of glycosides was recommended. Other Japanese workers have examined the far-i.r. spectra of mono-, di-, and tri-saccharides in the range 16—50 cm^{-1} at liquid-helium temperature; a number of absorption bands appearing in the spectra of di- and tri-saccharides were attributed to inter-ring interactions.[802]

Raman spectroscopy, which hitherto has been a largely neglected technique in carbohydrate chemistry, is beginning to be used more often. The Raman spectra of crystalline α-lactose and of lactose in aqueous solution have been recorded,[803] as have those of isocytosine in aqueous and deuterium oxide solutions.[804] It appears that laser-Raman spectroscopy can be a useful tool for identifying and investigating the different anomeric forms of sugars. Reference is made in Chapter 21 to Raman difference spectra of nucleosides complexed with the methylmercury(II) cation in water and in D_2O.

U.V. Spectroscopy

Vacuum-u.v. studies of a series of model compounds in the vapour phase have been undertaken to assist in an understanding of similar studies with monosaccharides

[798] R. Zhbankov, 'Infrared Spectra and Structure of Carbohydrates', Nauka i Tekhnika, Minsk, Beloruss. S.S.R., 1972.
[799] J. J. Cael, J. L. Koenig, and J. Blackwell, *Carbohydrate Res.*, 1974, **32**, 79.
[800] K. Morita, *Yakugaku Zasshi*, 1974, **94**, 739 (*Chem. Abs.*, 1974, **81**, 105 823w).
[801] K. Morita, *Yakugaku Zasshi*, 1974, **94**, 771 (*Chem. Abs.*, 1974, **81**, 105 900u).
[802] M. Hineno and H. Yoshinaga, *Spectrochim. Acta*, 1974, **A30**, 411 (*Chem. Abs.*, 1974, **80**, 146 431q).
[803] H. Susi and J. S. Ard, *Carbohydrate Res.*, 1974, **37**, 351.
[804] J. M. Delabar, *Z. Naturforsch.*, 1974, **29c**, 343.

(*cf.* Vol. 6, p. 177).[805] THF, tetrahydropyran, and several hydroxylated derivatives were found to absorb in the range 125—200 nm. 2-Hydroxytetrahydropyran was shown to exist mainly as the cyclic form in cyclohexane, but the hemiacetal function did not show the specific, high-wavelength absorption that might have been expected. The u.v. spectra of unsaturated aldonic acids have been examined for similar reasons (see next Chapter).

Mass Spectrometry

The mass spectrometry of carbohydrates has continued to attract attention, and variations of the electron-bombardment method have been used more frequently. Reviews on the applications of mass spectrometry to the structural analysis of naturally occurring carbohydrates [806] and to nucleosides and nucleotides [807] have appeared.

Space permits only a compilation of the compounds that have been studied specifically; other reports, concerned mainly with other aspects of the chemistry of carbohydrates, also contain mass-spectrometric information that is not included here. Detailed studies have been reported on the following classes of compounds: cyclopentene mono-ols, diols, and triols,[808] *O*-isopropylidene derivatives of tetrofuranoses and 5-deoxypentofuranoses,[809] aldoses, ketoses, lactones, and deoxy- and keto-sugars typically found among the products of radiolysis of sugars,[810] oleanolyl glycosides,[811] 2- and 4-mono- and 2,4-di-*O*-methyl ethers of methyl α-D-glucopyranoside,[812] TMS derivatives of acetylated methyl hexopyranosides,[813] methylated and acetylated derivatives of 5-thio-D-glucopyranose and related sulphur-containing sugars,[814] t-butyldimethylsilyl ethers of aldose and alditol derivatives,[175] and furylidene derivatives of D-glucose and D-xylose.[211a]

Nitrogen-containing derivatives studied by mass spectrometry include *N*-(2,4-dinitrophenyl) derivatives of amino-sugars and related alditols,[815] TMS derivatives of 2-acetamido-2-deoxyhexoses (specific peak intensities were used to distinguish between stereoisomers),[816] 2-acetamido-2-deoxy-α-D-glucopyranose tetra-acetate and analogues containing trideuterioacetyl groups,[817] acetylated derivatives of the methyl ethers of 2-deoxy-2-(*N*-methylacetamido)-D-glucitol,[156] *N*-acyldaunosamine derivatives,[818] and TMS derivatives of the methyl and trideuteriomethyl esters of *N*-acetylneuraminic acid.[819]

[805] H. R. Dickinson and W. C. Johnson, *J. Amer. Chem. Soc.*, 1974, **96**, 5050.
[806] L. Loenngren and S. Svensson, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 41.
[807] J. A. McCloskey, *Basic Principles Nucleic Acid Chem.*, 1974, **1**, 209.
[808] G. A. Singy and A. Buchs, *Helv. Chim. Acta*, 1974, **57**, 1158.
[809] A. Buchs, A. Glangetas, and J. M. J. Tronchet, *Helv. Chim. Acta*, 1974, **57**, 1333.
[810] M. Dizdaroglu, D. Henneberg, and C. von Sonntag, *Org. Mass Spectrometry*, 1974, **8**, 335.
[811] N. K. Kochetkov, A. F. Sviridov, L. P. Vecherko, V. I. Kadentsev, and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser khim.*, 1974, 113.
[812] V. Kováčik and P. Kováč, *Chem. Zvesti*, 1973, **27**, 662.
[813] H. B. Boren, P. J. Garegg, L. Kenne, A. Pilotti, S. Svensson, and C.-G. Swahn, *Acta Chem. Scand.*, 1973, **27**, 3557.
[814] V. Kováčik, P. Kováč, and R. L. Whistler, *Carbohydrate Res.*, 1973, **31**, 377.
[815] A. I. Usov, R. G. Krylova, S. N. Ryadovskaya, V. I. Kadentsev, and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2780.
[816] J. Vink, W. Heerma, J. P. Kamerling, and J. F. G. Vliegenthart, *Org. Mass Spectrometry*, 1974, **9**, 536.
[817] R. C. Dougherty, D. Horton, K. O. Philips, and J. D. Wander, *Org. Mass Spectrometry*, 1973, **7**, 805.
[818] A. Vigevani, B. Gioia, and G. Cassinelli, *Carbohydrate Res.*, 1974, **32**, 321.
[819] J. P. Kamerling, J. F. G. Vliegenthart, and J. Vink, *Carbohydrate Res.*, 1974, **33**, 297.

Derivatives of uronic acids examined by mass spectrometry include methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate and trideuteriomethylated derivatives thereof (providing a means of locating the substituent methyl groups)[820] and the corresponding uronamides,[821] and TMS derivatives of D-glucopyranosyluronic acid and the corresponding 6,3-lactone.[822]

Boronate esters have received a good deal of attention; studies include those on the mass spectrometry of benzene-, butane-, and methane-boronates of common carbohydrates as the TMS derivatives,[287] benzeneboronates of methyl 4,6-*O*-benzylidene-α-D-aldopyranosides containing vicinal *cis*-amino-alcohol groupings at C-2 and C-3,[288] and related esters of various aliphatic diols and triols.[284]

The retrodienic mode of cleavage can be used to locate the unsaturated linkage in alkyl glyc-2(3)-enopyranosid-4(2)-uloses.[823]

Acyclic compounds examined include 1,6-dibromo-1,6-dideoxy-D-mannitol,[824] and the peracetates of monosaccharide hydrazones[825] and aldono- and deoxy-aldono-nitriles.[826]

Chemical-ionization mass spectrometry has been used on several occasions. Horton and his co-workers have used both ammonia and isobutane as ionizing intermediates to obtain extremely simple spectra for a variety of carbohydrate derivatives.[827] In particular, $(M + NH_4)^+$ and $(M + H)^+$ ions were readily recognized with ammonia and isobutane, respectively, and a number of broad generalizations were made concerning the application of the technique. Chemical ionization with ammonia as the reagent gas can be used to obtain molecular weights for oligosaccharide acetates up to tetrasaccharides, and the glycosyl fragment ions in the spectra furnished unequivocal evidence about the nature of the non-reducing end of the oligosaccharide chain.[828, 829] Studies on lincomycin and its derivatives have suggested that chemical-ionization techniques can be used in several aspects of antibiotic research.[830]

Field-desorption mass spectrometry has been applied successfully to alditols, ketoses, and lactones,[831] oligosaccharides (up to tetrasaccharides),[832] glycosides,[833] and nucleosides and nucleotides.[834] The technique is particularly useful for determining molecular weights.

[820] V. Kováčik and P. Kováč, *Org. Mass Spectrometry*, 1974, **9**, 172.
[821] V. Kováčik and P. Kováč, *Carbohydrate Res.*, 1974, **38**, 25.
[822] M. L. McGregor, R. Reid, G. R. Waller, and E. C. Nelson, *Spectroscopy Letters*, 1974, **7**, 45.
[823] N. L. Holder and B. Fraser-Reid, *Tetrahedron*, 1973, **29**, 4077.
[824] M. Jarman and J. Tamas, *Org. Mass Spectrometry*, 1974, **8**, 377.
[825] V. Kováčik, K. Linek, and R. Sandtnerová, *Carbohydrate Res.*, 1974, **38**, 300.
[826] J. Szafranek, C. D. Pfaffenberger, and E. C. Horning, *Carbohydrate Res.*, 1974, **38**, 97.
[827] D. Horton, J. D. Wander, and R. L. Foltz, *Carbohydrate Res.*, 1974, **36**, 75.
[828] O. S. Chizhov, V. I. Kadentsev, A. A. Solov'yov, W. W. Binkley, J. D. Roberts, and R. Dougherty, *Doklady Akad. Nauk S.S.S.R.*, 1974, **217**, 362.
[829] R. C. Dougherty, J. D. Roberts, W. W. Binkley, O. S. Chizhov, V. I. Kadentsev, and A. A. Solov'yov, *J. Org. Chem.*, 1974, **39**, 451.
[830] D. Horton, J. D. Wander, and R. Foltz, *Analyt. Biochem.*, 1974, **59**, 452.
[831] M. M. Bursey and M. C. Sammons, *Carbohydrate Res.*, 1974, **37**, 355.
[832] J. Moor and E. S. Waight, *Org. Mass Spectrometry*, 1974, **9**, 903.
[833] W. D. Lehmann, H. R. Schulten, and H. D. Beckey, *Org. Mass Spectrometry*, 1973, **7**, 1103.
[834] H. R. Schulten and H. D. Beckey, *Org. Mass Spectrometry*, 1973, **7**, 861.

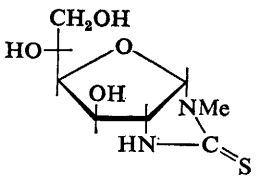
X-Ray Crystallography

The rate of application of *X*-ray crystallography has continued to increase, and the crystal and molecular structures of some 70 carbohydrates were determined during 1974. There is space to note only the names of compounds examined under the headings given below; solvent molecules of crystallization have not been included.

Free Sugars and Simple Derivatives thereof.—Bis(β-D-fructopyranose),$CaCl_2$,[835] β-lactose,[836] αα-trehalose, $CaBr_2$,[837] 1,2,3,4-tetra-*O*-acetyl-α- and -β-D-arabinopyranoses,[838, 839] 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose,[840] and 2,4-di-*O*-methyl-α-D-galactopyranose.[841]

Glycosides.—Methyl β-D-ribopyranoside,[842] methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside,[843] methyl 6-*O*-acetyl-β-D-glucopyranoside,[844] *trans*-*O*-β-D-glucopyranosyl methyl acetoacetate,[845] methyl 2,3,4,5-tetra-*O*-acetyl-α-D-glucoseptanoside,[846] methyl 1,5-dithio-α- and -β-D-ribopyranosides,[847] and ethyl 3-cyano-3,4-dideoxy-α-DL-*threo*-pentopyranoside.[848]

Amino-sugar Derivatives.—2-Acetamido-2-deoxy-α-D-galactopyranose,[849] 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (and the D-glucose analogue),[850] the bicyclic derivative (417) obtained from treatment of 2-amino-2-



(417)

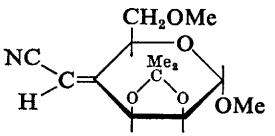
deoxy-D-glucose with methyl isothiocyanate,[851] 2-acetamido-2-deoxy-3-*O*-D-lactyl-α-D-glucopyranose (*N*-acetyl-α-D-muramic acid),[852] and the *m*-bromobenzyl glycoside of *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-α-D-neuraminic acid.[853]

[835] D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Cryst. Struct. Comm.*, 1974, **3**, 195.
[836] K. Hirotsu and A. Shimada, *Bull. Chem. Soc. Japan*, 1974, **47**, 1872.
[837] W. J. Cook and C. E. Bugg, *Carbohydrate Res.*, 1973, **31**, 265.
[838] V. J. James and J. D. Stevens, *Cryst. Struct. Comm.*, 1974, **3**, 187.
[839] V. J. James and J. D. Stevens, *Cryst. Struct. Comm.*, 1974, **3**, 19.
[840] V. J. James and J. D. Stevens, *Cryst. Struct. Comm.*, 1973, **2**, 609.
[841] I. C. M. Dea, P. Murray-Rust, and W. E. Scott, *J.C.S. Perkin II*, 1974, 105.
[842] A. Hordvik, *Acta Chem. Scand.*, 1974, **B28**, 261.
[843] V. I. James and J. D. Stevens, *Cryst. Struct. Comm.*, 1974, **3**, 27.
[844] P. J. Garegg, K. B. Lindberg, and C.-G. Swahn, *Acta Chem. Scand.* (*B*), 1974, **28**, 269.
[845] J. Ruble and G. A. Jeffrey, *Carbohydrate Res.*, 1974, **38**, 61.
[846] J. F. McConnell and J. D. Stevens, *J.C.S. Perkin II*, 1974, 345.
[847] R. L. Girling and G. A. Jeffrey, *Acta Cryst.*, 1974, **B30**, 327.
[848] B. P. Biryukov, B. V. Unkovskii, V. B. Mochalin, and A. N. Kornilov, *Zhur. strukt. Khim.*, 1973, **14**, 580.
[849] R. D. Gilardi and J. L. Flippen, *Acta Cryst.*, 1974, **B30**, 2931.
[850] L. T. J. Delbaere, *Biochem. J.*, 1974, **143**, 197.
[851] R. Jiménez-Garay, A. López-Castro, and R. Márquez, *Acta Cryst.*, 1974, **B30**, 1801.
[852] J. R. Knox and N. S. Murthy, *Acta Cryst.*, 1974, **B30**, 365.
[853] H. Wawra, *Z. Naturforsch*, 1974, **29c**, 317.

Acid Derivatives.—The trisodium salt of D-gluconic acid 6-phosphate,[854] the calcium-sodium complex of α-D-galactopyranosyluronic acid,[855] methyl (methyl α-D-galactopyranosid)uronate,[856] the barium salt of L-ascorbic acid 2-sulphate,[857] and calcium L-ascorbate.[858, 859]

A neutron diffraction study of potassium D-gluconate is mentioned in a later section.

Bicyclic Derivatives.—2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose,[860] 1,6-anhydro-3,4-*O*-isopropylidene-β-D-talopyranose,[861] methyl 3,4-*O*-ethylidene-β-D-galactopyranoside,[862] 3,4,6-tri-*O*-acetyl-1,2-*O*-[1-(*exo*-ethoxy)ethylidene]-α-D-glucopyranose,[863] 4,5-di-*O*-acetyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucoseptanose,[864] and the cyano-olefin (418).[865]



(418)

Other Derivatives.—2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl bromide,[866] 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl azide,[867] 2-*S*-ethyl-2-thio-D-mannose diethyl dithioacetal,[868] 1,4-anhydroerythritol,$NaClO_4$,[869] and *myo*-inositol 2-phosphate.[870] Inclusion complexes of cyclohexa-amylose with hydrogen peroxide[871] and with propan-1-ol[872] were also examined.

Nucleosides and Nucleotides and their Derivatives.—2,5′-Anhydro-2′,3′-*O*-isopropylideneuridine,[873, 874] 3′,5′-di-*O*-acetyl-2′-deoxy-2′-fluorouridine,[875] 1-β-D-

[854] G. D. Smith, A. Fitzgerald, C. N. Caughlan, K. A. Kerr, and J. P. Ashmore, *Acta Cryst.*, 1974, **B30**, 1760.
[855] J. Hjortas, B. Larsen, and S. Thanomkul, *Acta Chem. Scand.* (*B*), 1974, **28**, 689.
[856] J. Hjortas, B. Larsen, F. Mo, and S. Thanomkul, *Acta Chem. Scand.* (*B*), 1974, **28**, 133.
[857] B. W. McClelland, *Acta Cryst.*, 1974, **B30**, 178.
[858] R. A. Hearn and C. E. Bugg, *Acta Cryst.*, 1974, **B30**, 2705.
[859] J. Hvoslef and K. E. Kjellevold, *Acta Cryst.*, 1974, **B30**, 2711.
[860] F. Leung and R. H. Marchessault, *Canad. J. Chem.*, 1974, **52**, 2516.
[861] N. C. Panagiotopoulos, *Acta Cryst.*, 1974, **B30**, 1402.
[862] P. J. Garegg, K. B. Lindberg, and C.-G. Swahn, *Acta Chem. Scand.* (*B*), 1974, **28**, 381.
[863] J. A. Heitmann, G. F. Richards, and L. R. Schroeder, *Acta Cryst.*, 1974, **B30**, 2322.
[864] J. F. McConnell and J. D. Stevens, *Cryst. Struct. Comm.*, 1973, **2**, 629.
[865] G. Bernardinelli and R. Gerdil, *Helv. Chim. Acta*, 1974, **57**, 1459.
[866] P. Luger, P. L. Durette, and H. Paulsen, *Chem. Ber.*, 1974, **107**, 2615.
[867] P. Luger and H. Paulsen, *Chem. Ber.*, 1974, **107**, 1579.
[868] A. Ducruix and C. Pascard-Billy, *Acta Cryst.*, 1974, **B30**, 1056.
[869] R. E. Ballard, A. H. Haines, E. K. Norris, and A. G. Wells, *Acta Cryst.*, 1974, **B30**, 1590.
[870] C. S. Yoo, G. Blank, J. Pletcher, and M. Sax, *Acta Cryst.*, 1974, **B30**, 1983.
[871] P. C. Manor and W. Saenger, *J. Amer. Chem. Soc.*, 1974, **96**, 3630.
[872] W. Saenger, R. K. McMullan, J. Fayos, and D. Mootz, *Acta Cryst.*, 1974, **B30**, 2019.
[873] L. T. J. Delbaere and M. N. G. James, *Acta Cryst.*, 1974, **B30**, 1241.
[874] P. C. Manor, W. Saenger, D. B. Davies, K. Jankowski, and A. Rabczenko, *Biochim. Biophys. Acta*, 1974, **340**, 472.
[875] D. Suck, W. Saenger, P. Main, G. Germain, and J. P. Declercq, *Biochim. Biophys. Acta*, 1974, **361**, 257.

arabinofuranosyluracil,[876] 5,6-dihydro-2-thiouridine,[877] and uridine 5′-phosphate (disodium salt).[878]

The bis(pyridine)osmate complex of adenosine,[879] 8-aza-adenosine,[880] 2′,3′-dideoxy-2′,3′-didehydroadenosine,[881] and 9-β-D-arabinofuranosyladenine.[882, 883]

2′-Deoxyguanosine 5′-phosphate,[884, 885] guanosine 5′-phosphate and metal complexes thereof,[885–887] and guanosine 3′,5′-cyclic phosphate.[888]

Inosine 5′-phosphate [887, 889] and 2-ethylthio-8-methylinosine.[890]

6-Azacytidine [891, 892] and 1-β-D-arabinofuranosylcytosine.[893]

Two imidazoles have also been examined, *viz.* 1-*p*-chlorophenyl-4-*C*-(α-D-erythrofuranosyl)-4-imidazoline-2-thione [894] and (2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)imidazole (the acetyl groups were shown to be disposed axially due to the reverse anomeric effect).[732]

Antibiotic Substances.—Cytosamine triacetate,[895] 8,5′-anhydro-7-bromo-8-hydroxy-2′,3′-*O*-isopropylidene-tubercidin,[896] formycin hydrobromide,[897] and 2-methylformycin.[898]

Other Methods

Several unrelated reports have been grouped under this heading, each representing the application of techniques infrequently used in carbohydrate chemistry.

Neutron-diffraction studies of two forms of potassium D-gluconate have shown that they are conformational dimorphs, with one adopting a planar, zigzag conformation and the other geometrically related to it by rotation about the C-3—C-4 bond.[899]

[876] J. S. Sherfinski and R. E. Marsh, *Acta Cryst.*, 1974, **B30**, 873.
[877] B. Kojič-Prodič, R. Liminga, M. Šljukić, and Z. Ružić-Toroš, *Acta Cryst.*, 1974, **B30**, 1550.
[878] T. P. Seshadri, B. S. Reddy, and M. A. Viswamitra, *Current Sci.*, 1974, **40**, 339.
[879] J. F. Conn, J. J. Kim, F. L. Suddath, P. Blattmann, and A. Rich, *J. Amer. Chem. Soc.*, 1974, **96**, 7152.
[880] P. Singh and D. J. Hodgson, *J. Amer. Chem. Soc.*, 1974, **96**, 5276.
[881] W. L. B. Hutcheon and M. N. G. James, *Acta Cryst.*, 1974, **B30**, 1777.
[882] G. Bunick and D. Voet, *Acta Cryst.*, 1974, **B30**, 1651.
[883] A. K. Chwang, M. Sundaralingam, and S. Hanessian, *Acta Cryst.*, 1974, **B30**, 2273.
[884] D. W. Young, P. Tollin, and H. R. Wilson, *Acta Cryst.*, 1974, **B30**, 2012.
[885] T. P. Seshadri and M. A. Viswamitra, *Current Sci.*, 1974, **43**, 111.
[886] P. De Meester, D. M. L. Goodgame, T. J. Jones, and A. C. Skapski, *Biochem. J.*, 1974, **139**, 791.
[887] P. De Meester, D. M. L. Goodgame, T. J. Jones, and A. C. Skapski, *Compt. rend.*, 1974, **279**, *C*, 667.
[888] A. K. Chwang and M. Sundaralingam, *Acta Cryst.*, 1974, **B30**, 1233.
[889] N. Nagashima, K. Wakabayashi, T. Matzuzaki, and Y. Iitaka, *Acta Cryst.*, 1974, **B30**, 320.
[890] N. Nagashima and K. Wakabayashi, *Acta Cryst.*, 1974, **B30**, 1094.
[891] P. Singh and D. J. Hodgson, *Biochemistry*, 1974, **13**, 5445.
[892] P. Singh and D. J. Hodgson, *J. Amer. Chem. Soc.*, 1974, **96**, 1239.
[893] P. Tougard and O. Lefebvre-Soubeyran, *Acta Cryst.*, 1974, **B30**, 86.
[894] S. Pérez-Garrido, A. Conde, and R. Márquez, *Acta Cryst.*, 1974, **B30**, 2349.
[895] J. Sygusch, F. Brisse, and S. Hanessian, *Acta Cryst.*, 1974, **B30**, 40.
[896] K. Asami, K. Anzai, S. Suzuki, and H. Iwasaki, *Chem. Letters*, 1973, 1197.
[897] G. Koyama, H. Umezawa, and Y. Iitaka, *Acta Cryst.*, 1974, **B30**, 1511.
[898] J. E. Abola, M. J. Sims, D. J. Abraham, A. F. Lewis, and L. B. Townsend, *J. Medicin. Chem.*, 1974, **17**, 62.
[899] N. C. Panagiotopoulos, G. A. Jeffrey, S. J. La Placa, and W. C. Hamilton, *Acta Cryst.*, 1974, **B30**, 1421.

Mössbauer studies of a wide variety of Fe(III) complexes of mono- and polysaccharides have shown that they are polymeric, high-spin compounds in the solid state with co-ordination numbers of four (less usual), five, or six.[900]

L-Ascorbic acid radicals have been studied by e.s.r. methods in aprotic and aqueous media; the solvents were shown to affect the g factors and ^{1}H and ^{13}C hyperfine splittings.[513] Studies on the radicals resulting from irradiation of phenyl glycosides are referred to in Chapter 13.

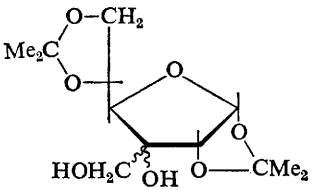
The binding of 2-acetamido-2-deoxy-α- and -β-D-glucopyranoses to lysozyme has been examined by calorimetry.[901]

[900] V. V. Khrapov, V. N. Kulakov, V. Kh. Shavratskii, R. A. Stukan, and V. I. Stanko, *Isotopenpraxis*, 1974, **10**, 227 (*Chem. Abs.*, 1974, **81**, 91 814e).
[901] A. Cooper, *Biochemistry*, 1974, **13**, 2853.

25
Polarimetry

Although polarimetry has fulfilled an important role since the early days of carbohydrate chemistry, it is fundamentally more complex than other physical methods. Several empirical treatments have permitted the relation between optical data and structure to be deduced, and a new development has been reported by Applequist.[902] The isotropic polarizabilities of all the atoms were included in computations of the optical rotations at 589 nm (sodium D-line) of a series of polyhydroxycyclohexanes in both the $^1C_4$ and $^4C_1$ conformations, and the results were compared with those obtained by calculations using Whiffen's rules. This approach represents a move away from empirical methods and is likely to produce results of considerable significance when applied to sugars and their derivatives.

New methods for determining the absolute configurations of alcohols and vicinal diols have been described. The addition of copper(II) hexafluoroacetylacetonate to solutions of monofunctional alcohols in carbon tetrachloride solution produced induced c.d. curves in the region 305—335 nm, the sign of which can be used to deduce the absolute chirality.[903] It was noted, however, that derivatized (*e.g.* acetylated or benzoylated) hydroxy-groups also gave induced dichroism, so that the results need be interpreted with care in such cases. A method involving the kinetic resolution of 2-phenylbutyric anhydride has been used in determining the absolute configurations of representative, cyclic and acyclic vicinal diols.[904] Such complexing reagents as nickel(II) acetylacetonate and $Pr(dpm)_3$ were also used for determining the absolute configurations of vicinal diols [*e.g.* (419)] and amino-alcohols.[905] Since the latter method can be applied in



(419)

[902] J. Applequist, *J. Amer. Chem. Soc.*, 1973, **95**, 8258.
[903] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1974, **96**, 4055.
[904] D. P. G. Hamon, R. A. Massy-Westropp, and T. Pipithakul, *Austral. J. Chem.*, 1974, **27**, 2199.
[905] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1974, **96**, 4057, 4059.

organic solvents, it is complementary to the Cupra-A method, on which further work has appeared.[906] The chirality of vicinal diamino-sugars can be established by c.d. measurements on the bis-*N*-(2,4-dinitrophenyl)ated derivatives.[907]

Other sugar derivatives on which o.r.d. and/or c.d. measurements have been made include 6- and 6′-mono- and 6,6′-di-*O*-tritylmaltose peracetates,[171a] methyl D-hexopyranoside acetates (the contributions from vicinal acetyl-oxygen functions were assigned),[908] mono- and di-saccharides containing the azide chromophore (the Cotton effects at *ca.* 280 nm were discussed in relation to the octant rule for azide derivatives),[909] glycosyloxy isoflavones (the Cotton effects were used to ascertain the positions of substitution),[910] 2,3-unsaturated aldonic acids (the signs of the Cotton effects at 250 and 210 nm were related to the configuration at C-4),[911] 1,6-anhydrodideoxy-β-D-*glycero*-hexopyranosuloses,[275] and 2-*C*-methylpentono-1,4-lactones (with a view to determining the stereochemistry at the chain branch).[912]

A useful review on the o.r.d. of nucleosides and nucleotides has appeared.[913] The c.d. spectra of uridine nucleosides have been measured in a variety of solvents, and the effect of solvents on the relative orientation of the sugar and base moieties has been discussed.[914]

[906] W. Voelter, H. Bauer, and G. Kuhfittig, *Chem. Ber.*, 1974, **107**, 3602.
[907] M. Kawai, U. Nagai, and T. Kobayashi, *Tetrahedron Letters*, 1974, 1881.
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[911] T. Sticzay, C. Peciar, S. Bystricky, and S. Kucar, *Chem. Zvesti*, 1974, **28**, 226.
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[913] T. L. V. Ulbricht, *Synthetic Proced. Nucleic Acid Chem.*, 1973, **2**, 177.
[914] A. Rabczenko, K. Jankowski, and K. Zakrzewska, *Biochim. Biophys. Acta*, 1974, **353**, 1.

26
Separatory and Analytical Methods

Chromatographic Methods

Gas–Liquid Chromatography.—In many cases, mass spectrometry of the individual components followed the separations noted below.

The examination of the following compounds by g.l.c. has been reported over the past year: TMS derivatives of lactose and sucrose,[915] aldononitrile acetates and alditol peracetates (using glass-capillary columns),[916] methylated aldoses (from polysaccharide hydrolysates) after reduction and either acetylation or trimethylsilylation,[917] and a variety of alditol esters (including heptafluorobutyryl esters).[918]

Glycosides to be examined by g.l.c. include those obtained from polysaccharide methanolysates (as the TMS derivatives),[919] methylated derivatives of methyl β-D-xylopyranoside (as the corresponding acetates),[920] mono-*O*-methyl ethers of methyl α-D-mannopyranoside (as the acetates),[921] tri-*O*-methyl ethers of methyl α-D-galacto-, -manno-, and -gluco-pyranosides (as the monoacetates),[922] and methylated derivatives of methyl L-gulopyranosides.[156a]

Trimethylsilylated *O*-methyloximes of 2-acetamido-2-deoxy-D-glucose, -mannose, and -galactose,[923] hexosamine trifluoroacetates,[924] and products (*e.g.* 2,5-anhydrohexoses) arising from the deamination of hexosamines[925] have also been examined by g.l.c.

The following acid derivatives have also been subjected to g.l.c.: alduronic and aldonic acids (as the acyclic oxime and/or ester TMS derivatives),[926] aldonic and alduronic acids (as deuterium-labelled alditol acetates),[927] methylated derivatives of methyl (methyl α-D-glucopyranosid)uronate (without derivatization and as TMS, acetate, and trifluoroacetate derivatives),[928] and conjugates (*e.g.* with 1-naphthol and androsterone) of β-D-glucuronic acid.[929]

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[920] E. V. Evtushenko and Yu. S. Ovodov, *J. Chromatog.*, 1974, **97**, 99.
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[922] Yu. N. El'kin, A. I. Kalinovskii, B. V. Rozynov, T. I. Vakorina, N. I. Shul'ga, and A. K. Dzizenko, *Khim. prirod. Soedinenii*, 1974, 451.
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[927] E. Sjöström, K. Pfister, and E. Seppälä, *Carbohydrate Res.*, 1974, **38**, 293.
[928] D. Anderle and P. Kováč, *J. Chromatog.*, 1974, **91**, 463.
[929] H. Ehrsson, T. Walle, and S. Wikström, *J. Chromatog.*, 1974, **101**, 206.

Intermediates in a synthesis of DL-streptose have also been analysed by g.l.c.[930]

Column and Ion-exchange Chromatography.—A systematic survey of the separations of reducing sugars, glycosides, and alditols by anion-exchange chromatography, covering the period 1962—1970, has been published.[931]

An interesting development has been the separation of the anomers of mono- and di-saccharides by anion-exchange chromatography at $-10\,^{\circ}C$,[932] while standard separations of reducing sugars,[933, 934] α-linked oligosaccharides of D-glucose,[935] and polyhydroxy-monocarboxylic acids[936] have been reported.

Paper Chromatography and Electrophoresis.—Reports on the chromatographic separations of neutral sugars[937] and tobramycin and related antibiotics[938] have appeared.

The behaviour of a series of aminocyclitols on electrophoresis in non-complexing and in complexing buffers has been studied[542] (see also Chapter 19).

Thin-layer Chromatography.—A permanent record of separations conducted on thin-layer chromatoplates can be obtained by xeroxing.[939]

A dilute acid solution of vanadium pentoxide has been used to detect carbohydrates on thin-layer chromatoplates.[940]

Two-dimensional chromatography on plates impregnated with sodium tetraborate has been advocated for the separation of clinically important sugars,[941] while more routine methods have been used in the chromatography of the anomeric phenyl D-ribo-furanosides and -pyranosides,[942] nucleosides (on chromatoplates coated with an anion-exchange resin),[943] and 2,2′-anhydrocytidine.[944]

High-pressure Liquid Chromatography.—A number of applications of this extremely useful technique have been reported during the past year; compounds examined include water-soluble polysaccharides and low-molecular-weight carbohydrates,[945] alditols and alkyl and phenyl glycosides,[946] phenolic D-glucuronides,[947] and nucleosides (using a packing of an anion-exchange resin).[948] High-pressure liquid chromatography has also been used in the analysis of novobiocin.[949]

[930] I. Stibor, J. Šrogl, and M. Janda, *J. Chromatog.*, 1974, **91**, 767.
[931] P. Jandera and J. Churáček, *J. Chromatog.*, 1974, **98**, 55.
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[934] A. M. C. Davies, D. S. Robinson, and R. Couchman, *J. Chromatog.*, 1974, **101**, 307.
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[937] M. C. Jarvis and H. J. Duncan, *J. Chromatog.*, 1974, **92**, 454.
[938] R. L. Hussey, *J. Chromatog.*, 1974, **92**, 457.
[939] R. Felici, E. Franco, and M. Cristalli, *J. Chromatog.*, 1974, **90**, 208.
[940] M. Malaiyandi, J. P. Barrette, and M. Lanouette, *J. Chromatog.*, 1974, **101**, 155.
[941] M. Ghebregzabher, S. Rufini, G. Ciuffini, and M. Lato, *J. Chromatog.*, 1974, **95**, 51.
[942] W. Z. Antkowiak and W. J. Krzyzosiak, *J. Chromatog.*, 1974, **90**, 399.
[943] J. Tomasz, *J. Chromatog.*, 1974, **101**, 198.
[944] R. L. Furner, J. D. Strobel, S. M. El Dareer, and L. B. Mellett, *J. Chromatog.*, 1974, **92**, 105.
[945] G. P. Belue and G. D. McGinnis, *J. Chromatog.*, 1974, **97**, 25.
[946] G. P. Belue, *J. Chromatog.*, 1974, **100**, 233.
[947] A. Assandri and A. Perazzi, *J. Chromatog.*, 1974, **95**, 213.
[948] P. R. Brown, S. Bobick, and F. L. Hanley, *J. Chromatog.*, 1974, **99**, 587.
[949] K. Tsuji and J. H. Robertson, *J. Chromatog.*, 1974, **94**, 245.

Counter-current Separations.—The separation and purification of several benzoylated D-ribofuranose derivatives have been accomplished by counter-current distribution between aqueous ethanol and mixtures of hexane and carbon tetrachloride.[950]

Other Analytical Methods.—Reagents used in the detection and determination of reducing sugars include the mercury(II)–edta complex (by measurement of the redox potential in alkaline solutions),[951] cerium(IV) sulphate (using both titrimetric[952] and fluorometric[953] monitoring), 2,2,5,5-tetrakis(carboxymethylthio)-*p*-dithian,[954] and *o*-toluidine (for distinguishing between hexoses and pentoses).[955]

A new fluorescence method for the detection of hexosamines is based on their conversion into diphenylindenone sulphonyl derivatives, which react with sodium ethoxide to give strongly fluorescent derivatives of diphenylisobenzofuran.[956] Unlike 2-acetamido-2-deoxy-D-glucose and -galactose, 2-acetamido-2-deoxy-D-mannose gave a yellow colour, having maximum absorption at 435 nm, under the normal conditions of the phenol–sulphuric acid method, although a negligible amount of colour was obtained from 2-amino-2-deoxy-D-mannose.[957] It is thus apparent that the estimation by this method of neutral sugars in polysaccharides containing 2-acetamido-2-deoxy-D-mannose can give rise to spurious results, unless suitable corrections are made.

Manual and automated spectrophotometric methods have been described for the determination of 3-deoxyhexonic (metasaccharinic) and 3-deoxy-2-*C*-hydroxymethylpentonic (isosaccharinic) acids.[958] Use was made of the chromophores formed by condensation of barbituric acid with the products of oxidation of the saccharinic acids with periodate, and the method obviates the need for solvent extraction required when 2-thiobarbituric acid is used in chromophore production.

Other oxidative methods of analysis are mentioned in Chapter 22.

[950] J. H. Vanbroeckhoven, *Bull. Soc. chim. belges*, 1974, **83**, 155.
[951] A. Abou El-Kheir and A. K. S. Ahmad, *Z. Lebensm.-Untersuch.*, 1974, **155**, 29.
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[953] S. Katz, W. W. Pitt, jun., J. E. Mrochek, and S. Dinsmore, *J. Chromatog.*, 1974, **101**, 193.
[954] G. Kunovits, *Analyt. Chim. Acta*, 1974, **70**, 213 (*Chem. Abs.*, 1974, **81**, 45 041c).
[955] A. I. Usov and S. V. Yarotskii, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 877.
[956] Y. Vladovska-Yukhnovska, Ch. P. Ivanov, and M. Malgrand, *J. Chromatog.*, 1974, **90**, 181.
[957] J. Hynan, G. Johnson, and Y. C. Lee, *Carbohydrate Res.*, 1974, **32**, 171.
[958] A. M. Y. Ko and P. J. Somers, *Carbohydrate Res.*, 1974, **33**, 281.

27
Alditols

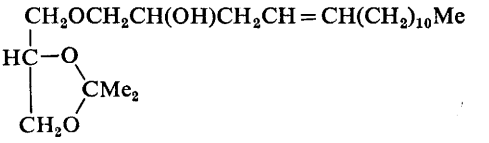
D-Mannitol has been isolated from *Peucedanum calcareum*, *Delphinium flexiosum*, *D. elisabethae*, *D. tamarae*, and *D. dzawachischvili*.[959] 1-*O*-Alkyl- and 1-*O*-(2-methoxyalkyl)-glycerols have been found in human colostrum, the milk of humans, cows, and sheep, and the red cells and plasma of sheep.[960] 1-*O*-(2-Methoxyalkyl)glycerols have also been isolated from lipids obtained from marine sources (herrings, mussels, and cod-liver oil, *etc.*), the main constituent having a long carbon-atom chain,[961] and 1-*O*-(2-hydroxyalkyl)glycerols containing chains of 14, 16, or 18 carbon atoms have been found in the liver oil of Greenland sharks.[962] A synthesized sample of 1-*O*-(2-hydroxy-*cis*-hexadec-4-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol (420) proved to be indistinguishable from the principal unsaturated component obtained from the latter source.

A brief review has appeared on the manufacture of D-glucitol (and D-mannitol) by the hydrogenation (and isomerization) of D-glucose.[963] Hydrolysis of the inter-saccharide linkage accompanied the high-pressure hydrogenation of sucrose over a ruthenium catalyst in the presence of phosphoric acid at 80 °C, giving a mixture of D-glucitol (85—87%) and D-mannitol (15—12%).[964] The kinetics of the hydrogenation of D-fructose over a nickel catalyst were shown to be modified by the presence of optically active tartaric and glutamic acids, and conditions affording a 66% yield of D-glucitol were reported.[965]

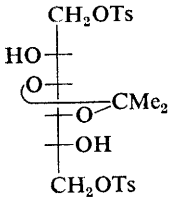
Improved syntheses of 2,3-di- and 2,3,6-tri-*O*-methyl-D-mannitol[164] and 1,2:5,6-di-*O*-isopropylidene-D-mannitol[966] have been described. The acid-catalysed reaction of 1,6-dichloro-1,6-dideoxy-D-mannitol with 1,1,1-trifluoropropanone furnished a mixture of diastereoisomeric 2,3:4,5-bisacetals, whose i.r. spectra and those of related fluorine-containing acetals were discussed.[967] Some reactions of derivatives of 1,6-di-*O*-toluene-*p*-sulphonyl-D-mannitol have been discussed in Chapter 4 (see Scheme 24), and it was also observed that syrupy 3,4-*O*-isopropylidene-1,6-di-*O*-toluene-*p*-sulphonyl-D-mannitol (421) was slowly transformed into 1,4-anhydro-2,3-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-D-

[959] G. E. Dekanosidze, A. Iarazashvili, D. G. Turabelidze, and E. P. Kemertelidze, *Khim. prirod. Soedinenii*, 1974, **10**, 79 (*Chem. Abs.*, 1974, **80**, 121 237t).
[960] B. Hallgren, A. Niklasson, G. Ställberg, and H. Thorin, *Acta Chem. Scand.* (*B*), 1974, **28**, 1029.
[961] B. Hallgren, A. Niklasson, G. Ställberg, and H. Thorin, *Acta Chem. Scand.* (*B*), 1974, **28**, 1035.
[962] B. Hallgren and G. Ställberg, *Acta Chem. Scand.* (*B*), 1974, **28**, 1074.
[963] L. W. Wright, *Chem. Technol.*, 1974, **4**, 42.
[964] N. A. Vasyunina and G. S. Barysheva, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 1587.
[965] M. A. Veksler, Yu. I. Petrov, N. G. Geling, and E. I. Klabunovskii, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 53.
[966] G. Kohan and G. Just, *Synthesis*, 1974, 192.
[967] G. M. Zarubinskii, B. Z. Volchek, and S. N. Danilov, *Zhur. obshchei Khim.*, 1973, **43**, 2760.

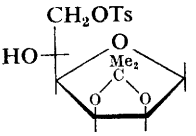
mannitol (422) on storage at room temperature by a reaction catalysed by adventitious toluene-*p*-sulphonic acid and involving either an intramolecular migration or an intermolecular trans-acetalation of the 3,4-*O*-isopropylidene group.[199] 1,3,6-Tri-*O*-toluene-*p*-sulphonyl-D-mannitol has been prepared by the selective sulphonylation of 3-*O*-toluene-*p*-sulphonyl-D-mannitol,[273] and the route shown in Scheme 132 has been used to synthesize the glycine derivative (423), which inhibited the neuraminidase present in *Vibrio cholerae*.[968]



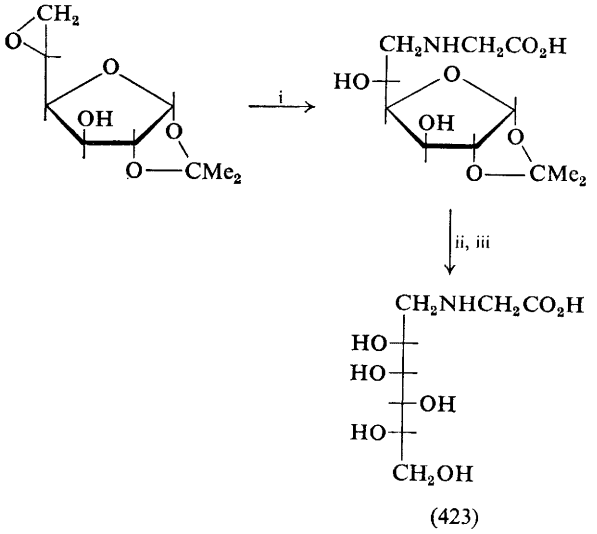
(420)



(421)



(422)



(423)

Reagents: i, $H_2NCH_2CO_2H$–$Ba(OH)_2$; ii, CF_3CO_2H; iii, $NaBH_4$

Scheme 132

[968] I. M. Privalova and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 216.

Syntheses of 3,6-anhydro-1-deoxy-1-(pyrimidin-1-yl)-D-glucitols [*e.g.* (365)][631] and -D(L)-galactitols[969] and related homonucleosides (see Chapter 21) have been reported. A series of standard transformations on D-[1,6-^{2}H]mannitol has afforded D-[3-^{2}H]glyceric acid 2,3-diphosphate.[249]

The most probable conformations of the heptitols and members of the higher alditols in solution have been deduced by Mills from stereomodels.[970] There are severe restrictions on the possible conformations adopted by higher alditols containing segments of *ribo* or *allo* configurations, for which it is impossible to construct models free from parallel 1,3-interactions. However, the higher members of the stereoregular series based on xylitol and iditol can adopt non-planar conformations free from serious non-bonded interactions. The conformations of alditols in aqueous solution have been indicated to be essentially the same as those of the corresponding acetates in chloroform solution.[519] The tendency for alditols to complex with metal cations was found to decrease in the order: *threo,threo* sequence > *threo* pair adjacent to a primary hydroxy-group > *erythro, threo* sequence > *erythro* pair adjacent to a primary hydroxy-group > *erythro, erythro* sequence. Since the formation of complexes was assumed to involve three consecutive oxygen atoms in a *gauche, gauche* arrangement, the energy required to produce this arrangement determined the extent to which complexes were formed. The latter and related[767] studies have also assessed the extent to which alditols complex with europium and praseodymium cations, respectively, in D_2O.

[969] A. Guarnieri, P. Roveri, G. Giovaninetti, T. Pozzo-Baldi, M. Amorosa, and J. Defaye, *Farmaco (Pavia) Ed. Sci.*, 1973, **28**, 862.
[970] J. A. Mills, *Austral. J. Chem.*, 1974, **27**, 1433.

Part II

MACROMOLECULES

1
Introduction

The main objectives of Part II remain unchanged from those of previous Reports in this series. The general policies adopted in Part II for naming micro-organisms, enzymes, and polysaccharides and commercial preparations thereof, and for the classification of enzymes were outlined in the Introduction to Volume 7, to which the reader is referred. The nomenclature of carbohydrate-degrading enzymes has been expanded slightly to indicate the configuration (D or L) of the sugar for which the enzyme is specific, whether or not the prefix is included in the recommended trivial name (see Vol. 7, p. 201).

Several recently reported carbohydrate-degrading enzymes have still to be assigned an E.C. number, and, in one or two instances, there appears to be some confusion over the definition of an enzyme in the latest Recommendations of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry on Enzyme Nomenclature. The description of 'chondroitin-sulphatase' ('chondroitin-sulphate sulphohydrolase', E.C. 3.1.6.4) is incorrect, based on the literature given in the Recommendations, although, since chondroitin-4-sulphate sulphatase is known, it is to be expected that chondroitin-6-sulphate sulphatase also exists (see Vol. 5, p. 378; Vol. 6, p. 489; Vol. 8, p. 385). The distinction between aspartylglucosylaminase ('1-L-β-aspartamido-2-acetamido-1,2-dideoxy-β-D-glucose amidohydrolase', E.C. 3.5.1.26) and '4-L-aspartylglycosylamine amidohydrolase' ['2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucosylamine', E.C. 3.5.1.37] is unjustified; careful reading of the literature cited in the Recommendations reveals that the names are synonymous (see also Vol. 4, p. 244; Vol. 6, p. 484; Vol. 7, p. 464; Vol. 8, p. 382).

Certain trends can be discerned from the literature over the past year or so. Although methodology has continued to be developed for elucidation of the structures of the carbohydrate moieties of macromolecules, significant advances have taken place in the deft application of existing methods, often on a very small scale, to investigations of plant, algal, and microbial polysaccharides, and glycoproteins and glycolipids. Work of this nature has revealed deficiencies of certain factors in a number of clinical conditions, the primary defects of which were hitherto unknown. The purification of many macromolecules has been aided by successful searches for suitable affinants and by the use of affinity chromatography.

Several new enzymes (*e.g.* a chitin deacetylase) have been reported, requiring prevailing theories of certain biosynthetic processes to be revised. The literature has also reflected a growing awareness of the need to relate the chemistry and biochemistry of life processes, particularly in humans.

Although laboratory syntheses of biologically active, carbohydrate-containing macromolecules are still a long way off, it is encouraging to find that there has been considerable progress towards this goal. For example, glycolipids and oligosaccharides have been synthesized (using new solid-phase techniques), pseudoglycoproteins possessing significant immunological properties have been obtained from the coupling of carbohydrates and proteins, and an enzymically active lysozyme has been synthesized. A start has also been made on exploring the applications of immobilized forms of such macromolecules as immunoglobulins and enzymes.

2
General Methods

BY R. J. STURGEON

Analysis

A new, non-corrosive reagent for automatic sugar chromatography with a sensitivity of 10^{-10} mol l^{-1} has been developed, using a dye based on the formation of a copper(I) complex of 2,2′-bicinchoninate.[1] The oxidizing capacity of the periodate complex of Cu^{III} has been used in the determination of a wide range of mono- and oligo-saccharides and organic acids.[2] Periodate ion does not interfere as an oxidant because Cu^{III}, being a stronger oxidant, reoxidizes any iodate that may be formed from the reaction between periodate ion and the substrate. An analytical procedure for the simultaneous, automated, quantitative analysis of reducing sugars, total carbohydrate, and starch in plants from a single sample has been presented.[3] The automated analysis of sugars, starch, and amylose in potatoes has been achieved by use of sugar–dinitrosalicylate and amylose–iodine colour reactions.[4] The initial oxidation of carbohydrates and the subsequent determination of the excess of periodate ion have been based on the observation that the reaction between periodate ion and hydrazine sulphate has a very high enthalpy change in both acidic and neutral media.[5] This method can be used for coloured solutions, which prevent the use of visual indicators; hence, it has a more direct use for the analysis of foodstuffs. Hexoses and pentoses can be measured singly or in combination with the *o*-dianisidine reagent, where the advantage of the high rate of reaction and the relatively low interference from hexoses allows the measurement of pentoses.[6] New methods have been reported for the determination of D-glucose, D-fructose, sucrose, maltose, and maltitol in foodstuffs by g.l.c. of the acetyl or TMS derivatives.[7] The separation, identification, and quantitative analysis of common fruit sugars, after removal of the non-volatile organic acids, have been achieved by using the TMS derivatives of the sugars.[8] Sugars and related hydroxy-acids have been determined, after separation, as the acyclic oximes or the ester TMS derivatives.[9] In a semi-micro, quantitative g.l.c. determination of carbohydrates in plant materials, the complete removal of

[1] K. Mopper and E. M. Gindler, *Analyt. Biochem.*, 1973, **56**, 440.
[2] P. K. Jaiswal and K. L. Yadava, *Indian J. Chem.*, 1973, **11**, 837.
[3] T. P. Gaines, *J. Assoc. Offic. Analyt. Chemists*, 1973, **56**, 1419.
[4] R. M. McCready, E. D. Ducay, and M. A. Gauger, *J. Assoc. Offic. Analyt. Chemists*, 1974, **57**, 336.
[5] L. S. Bark and P. Prachuabaibul, *Analyt. Chim. Acta*, 1974, **72**, 196.
[6] J. F. Goodwin and H. Y. Yee, *Clinical Chem.*, 1973, **19**, 597.
[7] Y. Iwata and Y. Sasaki, *J. Food Sci. Technol.*, 1973, **20**, 60.
[8] D. A. Heatherbell, *J. Sci. Food Agric.*, 1974, **25**, 1095.
[9] G. Petersson, *Carbohydrate Res.*, 1974, **33**, 47.

water was unnecessary when trimethylsilylimidazole was used as a silylating reagent.[10] Better resolution of hexoses from neutral glycolipids by g.l.c. of the alditol acetates has been achieved after standardization of the chromatographic conditions.[11]

New solvent systems and a new detector have been developed for the high-speed liquid-chromatographic separation of phthalic esters, carbohydrates, organic acids, and organic mercury compounds.[12] High-pressure liquid chromatography has been used in the separation of water-soluble polysaccharides from wood on columns of granular porous glass and polyacrylamide.[13] Sucrose, D-glucose, and D-fructose have been detected by differential refractometry, after elution from cation-exchange resins.[14] The separation of carbohydrates on ion-exchange resins has been achieved on a variety of ion-exchangers using water, aqueous ethanol, or aqueous solutions of non-complexing agents as eluants.[15] The extent of retardation of aqueous solutions of monosaccharides on alumina was reported to depend on its basicity.[16] For neutral alumina, the retardation was found to be proportional to the electrophoretic mobilities of the monosaccharides in alkaline arsenite. Monosaccharides containing three adjacent hydroxy-groups with either an *ax.*, *eq.*, *ax.*-orientation in the preferred pyranoid conformation, or possessing a high equilibrium concentration of furanoses, were strongly retarded. Monosaccharides arising from the hydrolysis of wood pulp have been determined quantitatively after t.l.c. separation on phosphate-impregnated Kieselguhr.[17] Solvent systems have been developed for the paper-chromatographic separation of a large number of monosaccharides, occurring in free or in bound form, in plants.[18] Liquid-scintillation counting of ^{14}C- and ^{3}H-labelled monomeric and polymeric carbohydrates, after separation by paper chromatography, was facilitated by the removal of water-soluble substances from the papers before assay.[19] The paper-electrophoretic mobilities of monosaccharides, methyl ethers, disaccharides, furanosides, uronic acids, and hexulosonic acids in glycerol–borate buffers were higher and the zones were sharper when compared with borate and sulphonated benzeneboronic acid buffers.[20] Chromatographic and m.s. methods for the separation and identification of sugars have been reviewed.[21]

The interference of 5-hydroxytryptamine in the assay of D-glucose by glucose oxidase:peroxidase chromogen-based methods has been demonstrated to result from competition between 5-hydroxytryptamine and other indoles, which can act as hydrogen donors to the peroxidase:peroxide system, and the chromogenic hydrogen donors.[22] A procedure has been described for the determination of

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D-glucose in serum or plasma, based on the fluorometric measurement of NADP(H) generated in the hexokinase method.[23] The measurement of D-glucose by methods based on oxidation with ferricyanide, 4-hydroxybenzoic acid hydrazide, glucose oxidase–*o*-toluidine, and glucose oxidase and an oxygen electrode have been compared.[24] Radioactive D-glucose has been measured by the enzymic removal of 1-^{14}C as $^{14}CO_2$ in a method that incorporates a system for recycling NADP.[25] A cerimetric measurement of D-glucose has been developed incorporating the reduction of a bivalent copper salt in alkaline solution, transfer of the resulting copper oxide into acid, and potentiometric titration of copper with a ferroin indicator.[26] Developments in the enzymic determinations of D-glucose and its anomers have been reported.[26a]

A modified resorcinol procedure has been used to give increased specificity in the determination of D-fructose in the presence of D-glucose and D-glucose phosphates.[27] Harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole) has been used in the quantitative determination of hexuronic acids; pentoses react only after the addition of cysteine, thereby allowing the determination of small amounts of pentoses in the presence of larger amounts of hexuronic acids and other sugars.[28] Aldonic acids, deoxyaldonic acids, hexuronic acids, and hexonolactones have been analysed by g.l.c. as mixtures of the lactone TMS ethers and the linear ester TMS ethers.[29] A simplified procedure has been used for the determination of uronic acids by acidic decarboxylation in a gas-absorption apparatus.[30] After reduction with sodium borodeuteride, mixtures of D-gluconic and D-glucuronic acid lactones have been determined quantitatively as the alditol acetates by g.l.c.–m.s.[31] Formation of borate complexes has been shown to contribute significantly to the distribution coefficients obtained in the separation of polyhydroxy-acids in borate media.[32] This contribution was shown to increase with the number of vicinal hydroxy-groups and with their distance from the carboxylic acid group. Vicinal hydroxy-groups in the *gauche*, but not in the *anti*, configuration have the ability to form strong complexes, provided that the steric conditions are favourable in other respects. A spectrophotometric method for the determination of sialic acids in glycoproteins and glycopeptides is based on the formation of a complex between the sialic acid and 1,10-phenanthroline.[33] A gel-permeation chromatographic procedure has been employed in a separation of 2-amino-2-deoxy-D-glucose from the corresponding alditol.[34] Amino-sugars in body fluids have been separated as the trifluoroacetate derivatives in a rapid

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g.l.c. procedure.[35] Contrary to previous reports, 2-acetamido-2-deoxy-D-mannose, but not 2-amino-2-deoxy-D-mannose, gives a colour reaction with phenol–sulphuric acid; under identical conditions, negligible reactions are given by 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose.[36] A polyamide liquid phase has been used in an effective separation of 2-amino-2-deoxy-D-glucose, -D-galactose, and -D-mannose.[37] 2,5-Anhydro-D-mannose, 2,5-anhydro-D-talose, and D-glucose, formed by deamination of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and 2-amino-2-deoxy-D-mannose, respectively, have been derivatized as the aldononitrile acetates and determined by g.l.c.[38] A single-column procedure for the programmed analysis of the amino-acids of collagen and basement membrane was able to separate 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose.[39]

Differential refractometric and u.v. detectors have been employed in the analysis of polyhydric alcohols following separation by high-pressure liquid chromatography on columns of silica gel.[40] A method for the spectropolarimetric analysis of mixtures of the molybdate complexes of D-glucitol and mannitol in the presence of D-glucose has been described.[41] When the optical rotation of molybdate complexes of D-glucitol and mannitol is measured at low pH values, only the mannitol content is determined.[42] A t.l.c. technique, with a sensitivity comparable to g.l.c., has been used for the rapid estimation of traces of mannitol in D-glucitol, and *vice versa*.[43] The colorimetric estimation of 3-deoxy-D-*manno*-octulosonic acid (KDO), in free form and bound in oligosaccharides, has been developed, using a diphenylamine reagent.[44]

Maltose phosphorylase from *Lactobacillus brevis* has been shown to be highly specific in its arsenolytic reaction for maltose yielding D-glucose, which may be measured on coupling to the glucose oxidase procedure.[45] Malto-oligosaccharides (containing up to fifteen D-glucose units) and polymaltotrioses (with chain lengths up to 21) have been fractionated by gel chromatography.[46] An automated hypoiodite-oxidation procedure has been applied to the analysis of oligosaccharides in column eluates.[47] Oxidation of the aldoses and substituted aldoses gave the corresponding aldonic acids, which liberated glyoxylic acid and formaldehyde on periodate oxidation, thus enabling 3-, 4-, and 6-*O*-substituted aldonic acids to be distinguished. An automated system using an anion-exchange resin has been developed for the separation and quantitation of the α-linked oligosaccharides of D-glucose derived from dextran.[48] Oligomeric sugar alcohols

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have been separated by partition chromatography on ion-exchange resins.[49] The solubility of higher oligomers in aqueous ethanol limits the practical range of application to species with a DP *ca.* 10. A direct enzymic procedure for the assay of liver glycogen involves treatment with amyloglucosidase, followed by determination of the liberated D-glucose with glucose oxidase.[50] An automated enzymic procedure for the estimation of a number of oligoglucans has been developed.[51] Malto-oligosaccharides were degraded by a combination of β-amylase and α-glucosidase, whereas laminarisaccharides were cleaved with yeast β-glucosidase; in both cases, the derived D-glucose was estimated with glucose oxidase. Celite coated with polymeric 1,3-diaminobenzene has been used for the selective adsorption of glycogen and other branched polysaccharides, whereas neutral and charged monosaccharides were not adsorbed.[52] The influence of inorganic and organic salts on the bonding of polysaccharides with thiazine dyestuffs has been discussed.[53] Incorporation of tritium has been used for the quantitation of uronic acids in polysaccharides, by allowing them to react with 1-cyclohexyl-3-(2-morpholinoethyl)carbodi-imide metho-*p*-toluenesulphonate and then with sodium borotritide.[54] Sphingosine and 2-amino-2-deoxyhexoses have been shown to react with fluorescamine (4-phenylspiro[furan-2(3*H*),1′-phthalan]-3,3′-dione).[55] Fatty acids, fatty-acid aldehydes, phospholipids, glycolipids, and cholesterol have been determined after staining with Malachite Green.[56] An improved method for the automated determination of meta- and iso-saccharinic acids has utilized the chromophores formed by condensation of barbituric acid with the products derived from oxidation with periodic acid.[57] A high-resolution periodic acid–Schiffs reagent has been devised for the determination of glycoproteins, after their separation by polyacrylamide gel electrophoresis.[58] Increases in staining intensity and in band resolution were achieved. Mixtures of such carbohydrates as glycogen, amylopectin, and branched-chain polysaccharides, which cannot be separated by complex formation with concanavalin A, may be separated by use of an immobilized form of concanavalin A.[59] A zymogram technique for the detection of carbohydrases, after electrophoretic separation, has been reported.[60] Crossed immuno-affinoelectrophoresis has been described as an analytical technique; the principles of electrophoresis and biospecific interactions are combined in this technique, which allows the results of affinity-chromatographic experiments to be predicted.[61] The possible identification of such components as glycoproteins, with certain recognizable molecular structures, has been suggested. A modification of the Nash procedure for the

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determination of formaldehyde has been reported.[62] Small amounts of formaldehyde, liberated during oxidation of sialoglycoproteins, have been estimated in a reaction with 3-methyl-2-benzothiazolone hydrazone.[63]

Structural Methods

Several of the latest methods and techniques used in structural polysaccharide chemistry have been reviewed;[64] these included methylation analysis, partial hydrolysis, various specific methods of degradation, and g.l.c.–m.s. A new method for the specific degradation of polysaccharides involves the preparation of methylated polysaccharides containing a number of free hydroxy-groups at defined positions, oxidation of these to carbonyl groups with ruthenium tetroxide, and subsequent β-elimination by treatment with base.[65] Analyses of the degradation products provided evidence concerning the sequence of sugar residues in the original polysaccharide. Permethylated dicarbonyl sugars have been prepared from a variety of methylated polysaccharides.[66] The methyl tri-*O*-methyl-D-glucopyranosides obtained from permethylated cellulose, laminarin, and dextran were oxidized with DMSO–phosphorus pentoxide to give methyl 2,3,6-tri-*O*-methyl-D-*xylo*-hexopyranosid-4-ulose, methyl 2,4,6-tri-*O*-methyl-D-*ribo*-hexopyranosid-3-ulose, and methyl 2,3,4-tri-*O*-methyl-D-*gluco*-hexodialdo-1,5-pyranoside, respectively. The chromatographic and spectrophotometric properties of the 2,4-dinitrophenylhydrazones of these compounds were investigated. The linkage analysis of (1 → 6)-linked oligosaccharides by degradation with alkali has been reported.[67] 2-*C*-Methylglyceric acid was formed by sequential degradation of the chain in isomaltose oligosaccharides, and products of the stopping reaction, *viz.* 6-*O*-substituted 3-deoxy-D-hexonic acids, were also obtained. Deuteriated 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic and 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acids, 3-deoxy-D-*ribo*-hexonic and 3-deoxy-D-*arabino*-hexonic acids, and 2-*C*-methyl-D-ribonic acid have been isolated from the degradations of maltose, turanose, and inulin, respectively, in aqueous barium deuteroxide.[68] The position and extent of incorporation of deuterium in these products were examined by using m.s. and ^{1}H n.m.r. techniques. Saccharinic acids isolated after using aqueous barium tritioxide in an atmosphere of nitrogen were found to contain carbon-bound tritium, whilst a similar degradation of maltose in the presence of air gave, in addition, *O*-D-glycosylaldonic acids that were not tritium-labelled.[69] The application of this procedure to structural analysis of oligosaccharides was discussed.

A review of the ^{1}H n.m.r. spectroscopy of oligo- and poly-saccharides has been published.[70] The complete assignment of the ^{13}C and ^{1}H n.m.r. spectra of

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permethylated α- and β-D-galactopyranosides has been accomplished with the aid of specific trideuteriomethylation, heteronuclear spin-decoupling, and computer simulation of the spectra.[71] Identification of partially methylated D-galactoses, *e.g.* as obtained from the methylation of galactans, was carried out by conversion of the free hydroxy-functions into ^{2}H- or ^{13}C-labelled methoxy-groups, and comparison of the n.m.r. spectra of the resulting permethyl ethers with those of reference compounds. By comparison with methyl α- and β-L-arabinofuranosides, the L-arabinofuranosyl residues in an arabinogalactan from coffee beans have been shown to have the α-configuration.[72] On the basis of ^{1}H n.m.r. spectroscopic data for the TMS derivative of β-D-galactofuranose, it was concluded that the molecule occurs mainly in the ^{4}E(D) envelope conformation. In the preferred conformation of the C-4—C-5—C-6 chain, H-4 and O-5 and also O-5 and O-6 are in *trans*-coplanar arrangements.[73] The complete interpretation of the high-resolution ^{1}H n.m.r. spectra and accurate chemical shifts and coupling constants, obtained after computer simulation of the spectra, of the TMS derivatives of six 2-acetamido-2-deoxy-D-aldohexopyranoses have been reported.[74] All pyranoid rings were shown to occur in the $^{4}C_{1}$(D) chair conformation, but the preferred conformation of the C-5-CH_2OTMS group depends on the configuration at C-4. A similar study was undertaken with the TMS derivatives of a number of 6-deoxyaldohexopyranoses, of β-D-altro-, β-D-allo-, and α- and β-D-talo-pyranoses,[75] and of D-fructoses and oligosaccharides containing β-D-fructofuranosyl residues.[76] Rules were presented for estimation of the chemical shifts of the ring protons of TMS derivatives of aldohexopyranoses and 6-deoxyaldohexopyranoses. The ^{1}H n.m.r. spectra of D-gluco-oligosaccharides and D-glucans have been studied with respect to the anomeric proton.[77] In (1 → 2)-linked disaccharides, the effect of change in configuration of the hydroxy-group at C-1 on the chemical shifts of the glycosidic proton was noted. Equilibrium mixtures of (1 → 2)-linked disaccharides were found to contain more α-anomer than did the other examples, despite the *cis* configuration of substituents at C-1 and C-2. Some D-glucans were investigated with regard to the degrees of branching. Peaks assigned by correlation of the ^{13}C n.m.r. spectra of permethylated disaccharides with those of the constituent, permethylated monomers indicated large shift-increments for skeletal and methoxyl carbons; these were explained in terms of steric or proximity effects.[78] The configuration of the glycosidic linkages could be deduced from the chemical shifts of the anomeric carbons and from the ^{1}H n.m.r. data of the attached protons. Interpretation of the ^{1}H n.m.r. spectra of mono-, di-, tri-, tetra-, and penta-D-galactopyranuronic acids, the corresponding fully esterified

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methyl esters, the partly esterified di- and tri-D-galactopyranuronic acids, and unsaturated di-, tri-, and tetra-D-galactopyranuronic acids has been reported.[79] The site of esterification in the non-reducing or the reducing residues could be deduced, and it was confirmed that all the D-galacturonic acid residues assume the $^4C_1$ conformation and are α-(1 → 4)-linked. In the unsaturated oligo-D-galactopyranuronic acids, the double bond is located between C-4 and C-5 of the sugar unit at the non-reducing end, with the 4-deoxyhex-4-enopyranosyluronic acid residue occurring in the $^2H_1$(D) conformation.

The significant advances made in the use of mass spectrometry in the structural analysis of neutral carbohydrates have been reviewed.[80] The characterization of benzene-, butane-, and methane-boronate TMS derivatives of some common carbohydrates has been investigated by a combination of high- and low-resolution m.s.[81] Preparation of stereospecific derivatives with alkane- or arene-boronic acids, followed by silylation and m.s., allowed the identification of isomeric structures of pentoses, hexoses, 6-deoxyhexoses, and 2-acetamido-2-deoxyhexoses. The mass spectra of per-*O*-acetylaldono- and per-*O*-acetyldeoxyaldono-nitriles have been recorded, with the major fragmentation pathways described in terms of the application of electron-impact m.s. to structural studies of aldoses.[82] Both acetyl and deuteryl-labelled acetyl derivatives showed that the spectra are useful in verifying the position(s) of deoxy-group(s) in deoxyaldoses. The number and positions of the *O*-acetyl groups in the 2-, 3-, 4-, 6-, 2,3-, 3,6-, and 4,6-positions of TMS ethers of methyl D-hexopyranoside acetates have been unequivocally determined by m.s.[83] The separation and structural determination of ten partially methylated methyl D-glucosides have been achieved by g.l.c.–m.s. studies.[84] Information has been obtained, from g.l.c.–m.s. of the TMS derivatives, on the products of alkaline degradation of 3,4-di- and 3,4,6-tri-*O*-methyl-D-glucoses.[85] During reaction with alkaline sodium borohydride, reduction occurred more rapidly than β-elimination, and the only detectable products were the corresponding alditols and epimeric 3-deoxyalditols. Reaction with the base alone, followed by reduction, gave mixtures of aldonic acids, including the epimeric 3-deoxy-4-*O*-methylaldonic acids (metasaccharinic acids), 3-deoxyaldonic acids (arising from the loss of the 4-*O*-methyl substituent), and 3,4-dideoxyaldonic acids. Fragmentation pathways for methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronamide have been proposed from a study of compounds specifically labelled with CD_3 and ND_2 groups.[86] The presence of an amino-group in the molecule was found to give rise to a new series of fragmentations. Partially methylated derivatives of hexuronic acids, obtained by methylation analysis, can be identified by exhaustive trideuteriomethylation and conversion into crystalline amides. From a study made of the m.s. of a number of model carbohydrates as acetates of the *N*-phenylosotriazole derivatives, a successful method

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has been developed for determining the sequence of monosaccharide units in oligosaccharides.[87] Methyl ethers of 2-deoxy-2-(*N*-methylacetamido)-D-glucose have been separated and identified by g.l.c.–m.s.[88] The (2 → 3)- and (2 → 6)-linked isomers of *N*-acetylneuraminyl-lactose have been differentiated by means of the characteristic fragmentation patterns obtained on m.s. of their TMS derivatives.[89] In a sequence analysis of complex oligosaccharides by m.s., a comparison has been made of the per-*O*-methyl and per-*O*-TMS derivatives of lacto-*N*-tetraose.[90] The use of per-*O*-methyl derivatives offers the advantage of smaller molecular weights, higher volatility, and stability to solvolysis, but, despite the higher molecular weights, the per-*O*-TMS derivatives gave fragments of relatively low molecular weight that are diagnostic of important molecular features. Molecular ions have been obtained on the m.s. of glycosphingolipid derivatives containing up to six sugar units.[91] The spectra of the reduced derivatives showed abundant ions containing the complete carbohydrate moiety, thus allowing conclusions to be drawn regarding the carbohydrate composition (including the ratios of hexose, hexosamine, and sialic acid). Conclusive information was also obtained on the carbohydrate sequence (including chain branching). In a micro-preparative g.l.c. method, a separation of the products of partial methylation of methyl β-D-xylopyranoside has been obtained.[92]

Comparative studies of the kinetics have shown that the acetolysis reaction rates for various β-linked D-glucose disaccharides decrease in the order (1 → 6) ⩾ (1 → 3) > (1 → 2) > (1 → 4).[93] For the corresponding α-linked disaccharides, the order was (1 → 6) ⩾ (1 → 4) > (1 → 3) > (1 → 2), whereas for D-mannose disaccharides the order was α-(1 → 6) ⩾ α-(1 → 3) > β-(1 → 4) > α-(1 → 2). A reaction mechanism has been proposed which features an acyclic intermediate, and, for certain disaccharides, anchimeric assistance by the C-2 acetoxy-group. The biological functions of skeletal polysaccharides have been correlated in a simple way with their conformations.[94] A procedure for the simultaneous optimization of bond lengths and angles has been used to test different models for mannan I.[95]

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3
Plant and Algal Polysaccharides

BY R. J. STURGEON

Introduction

A number of review articles have been published on plant polysaccharides under the following titles: Primary Cell Wall and Control of Elongation Growth,[1] Site of Synthesis of Polysaccharides of the Cell Wall,[2] Biosynthesis of Pectin and Hemicelluloses.[3] A review of food technology has dealt with the manufacture and application of edible gums and related substances.[4]

Starch

'New Directions for Starch and Glucose Research' was used as the title of a review on the use of enzymes for the modification of starch.[5] Methods dealt with include the conversion of starch into D-glucose, D-glucose and D-fructose, and glycoproteins, as well as the modification of starches with high degrees of branching. Some aspects of the enzymic degradation,[6] and the structure and metabolism[7] of starch have been reviewed. A review of current concepts of starches of high amylose content has dealt with studies from genotypes of pea, maize, and barley which are distinguished by abnormal granular forms and by the presence of unusual starch material.[8] The molecular properties of starch components and their relation to the structure of the granule have been reported.[9] The results implied that, although the biosynthesis of amylose and amylopectin may follow the same general pattern in a number of plants, the subsequent process of the packing together of the two polysaccharides to form the starch granule may be different in each species or even in varieties within a given species.

A much more accurate estimation of amylose has been claimed for the use of a double-wavelength method on the iodine complex.[10] Low concentrations of the amylose–iodine complex, in general, have been found to be insoluble in high concentrations of calcium chloride.[11] However, the hydroxyethylamylose–iodine

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[2] D. H. Northcote, in ref. 1, p. 165.
[3] H. Kauss, in ref. 1, p. 191.
[4] R. F. Greenwood, *Chem. and Ind.*, 1974, 657.
[5] R. L. Whistler, *Stärke*, 1974, **26**, 334.
[6] D. J. Manners, in ref. 1, p. 109.
[7] D. J. Manners, in 'Essays in Biochemistry', ed. P. N. Campbell and F. Dickens, Academic Press, London and New York, 1974, p. 37.
[8] W. Banks, C. T. Greenwood, and D. D. Muir, *Stärke*, 1974, **26**, 257.
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[10] M. Sanyal, V. S. Rao, and K. B. De, *Z. analyt. Chem.*, 1974, **271**, 208.
[11] F. R. Dintzis, *Stärke*, 1974, **26**, 56.

complex is soluble and stable in this reagent, although the colour-stability of some of the complexes was observed to be time-dependent. Optimum conditions have been reported for the quantitative hydrolysis of starch and glycogen to D-glucose by acid, in which no significant levels of acid-catalysed reversion are produced.[12] The relative ease of hydrolysis of the α-(1 → 6)-bonds in branched glucans was also investigated. Quantitative determinations of starch and glycogen, and their metabolism in the leaves of *Tussilago farfara* during infection by *Puccinia poarum*, have been achieved by use of the amyloglucosidase–glucose oxidase procedure.[13]

A suitable method has been described for the isolation of high-molecular-weight amylose and amylopectin from cereal and tuber starches by dissolution of the granules in urea.[14] Fractionation of starch into amylose and amylopectin has been achieved by preferential precipitation of amylose with octyltrimethyl-ammonium bromide.[15] By use of [^{14}C]amylose and unlabelled amylopectin, and *vice versa*, a complete separation of the polysaccharides was demonstrated. Optimum conditions have been reported for the ethanol-induced adsorption of amylose from starch on to columns of cellulose used in the purification of amylopectin.[16]

Acetaldehyde, methanol, and acetone have been found to be the main volatile components formed during the γ-irradiation of maize starch.[17] The effects of radiation were compared to those of heat treatment, and the effects of concentration were followed during storage. Oxidation of wheat starch with sodium periodate was enhanced after γ-irradiation, as indicated by the formation of greater amounts of formic acid from the irradiated samples.[18] Destruction of amylopectin was considered to occur with the formation of small, branched fragments. Formic acid represented the main part of the free acidity generated by irradiation of maize starch.[19] Treatment of Egyptian sweet-potato starch with different doses of γ-rays caused an increase in reducing sugars, as well as molecular degradation, resulting in a marked decrease in viscosity and of water absorption; the solubility increased on irradiation.[20]

The results of kinetic and hydrodynamic studies of the amylose–iodine reaction have been analysed in an attempt to resolve the controversy surrounding the conformation of amylose in dilute, aqueous solution.[21] Intensity calculations using computer models have confirmed that there are six residues per turn in the monohydrated, helical amylose polymorph.[22] Comparison of the results with those obtained for V-amylose dihydrate indicated no major conformational differences between the two helices. A net helical rotation of about 30° accompanied the monohydrate–dihydrate transition, and the rotational position in the

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[18] M. A. Abd Allah, Y. H. Foda, and R. El Saadany, *Stärke*, 1974, **26**, 89.
[19] J. F. Dauphin, H. Athanassiadis, G. Berger, and L. Saint-Lèbe, *Stärke*, 1974, **26**, 14.
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[22] B. Zaslow, V. G. Murphy, and A. D. French, *Biophysics*, 1974, **13**, 779.

monohydrate allowed packing that was less close. Unit-cell dimensions from *X*-ray diffraction patterns of amylose–fatty acid complexes have been calculated for both wet and dry states.[23] Both six- and seven-helical conformations of amylose were found in the complexes, with the conformation appearing to depend on the linear chain length of the fatty acid molecules. β-Amylase limit dextrins of amylopectin and glycogen have been completely debranched by the joint actions of isoamylase and pullulanase.[24] The relative numbers of A(unsubstituted)- and B(substituted)-chains of the dextrins and the native polysaccharides were calculated; amylopectin was shown to contain twice as many A- as B-chains. The techniques of semi-micro-differential potentiometric titration of starch with iodine and colorimetric assay of an aqueous starch–iodine solution have been compared as analytical tools for the measurement of the amylose content of starches.[25] Although the colorimetric method could be calibrated to give results comparable to the potentiometric assay, the former technique was found to be inapplicable to the assay of maize starches of high amylose content. The iodine-staining properties of a range of maltosaccharides ($\overline{DP}$ 6-22) have been shown to rise initially, with a logarithmic increase in the intensity of absorption followed by a linear relationship.[26] A plot of the reciprocal of the wavelengths of maximum absorbance against $\overline{DP}$ indicated the existence of three separate linear sections corresponding to $\overline{DP}$'s 6-11, 12-18, and 18-22. Prolonged treatment of granular potato starch with acid to produce a lintnerized starch was found to occur in two stages; the first stage is attributed to hydrolysis of the amorphous parts of the granules, and the second stage to hydrolysis of the more organized areas.[27] At the same time, there was a progressive appearance of two soluble, major-chain populations, one being linear and of $\overline{DP}$ 15, and the other being singly branched and of $\overline{DP}$ 25. Insoluble starch particles occurring in acid-thinned, corn-starch hydrolysates have been identified as amyloses in degraded and associated forms, whereas the same particles in enzyme-thinned hydrolysates were complexes of degraded amylose and free fatty acids.[28] Under ordinary analytical conditions, it has been shown that the proportion of periodate-resistant D-glucosyl residues in starches and glycogens is consistently about one-third of the proportion of branch points.[29] The resistant D-glucosyl residues became freely oxidizable after the limit-oxidized glucans had been reduced with sodium borohydride. It is assumed that, when a D-glucosyl residue carrying a branch at position 6 is oxidized, the resulting two aldehyde groups both form six-membered hemiacetal rings with the closest hydroxy-groups on neighbouring, unoxidized residues in the same (1 → 4)-linked chain, whereas when the other D-glucosyl residues are oxidized, only one of the aldehyde groups shows a strong tendency to form a hemiacetal of this kind. It is suggested that, in the unbranched units, the other aldehyde group preferentially forms a hemiacetal with the primary hydroxy-group in the same unit.

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The enzymes of starch biosynthesis in developing and mature kernels of normal dent corn, and the effects of several endosperm mutations on the carbohydrate composition of the kernel have been reviewed.[30] An investigation has been reported on the effects of different conditions and metabolites on the starch content of leaf strips of *Zea mays* in relation to the regulation of starch metabolism.[31] The role of an unprimed phosphorylase in the biosynthesis of starch has been discussed,[32] and a review has appeared of glycogen plastid differentiation in Müllerian body cells of *Cecropia peltata*.[33] Polyglucoside synthesis *via* the starch phosphorylase-β-amylase complex in diverse Saccharum genotypes,[34] and some fundamental problems in the biosynthesis of starch granules[35] have been reviewed. In normal cultivars of barley and wheat, the amylose content of starch was shown to be independent of the size of the granule.[36] Branching with Q-enzyme using amylose as a carrier gave a more-or-less branched amylopectin-like substance, whereas chemical branching furnished comb- or star-like structures.[37] Investigations on the mode of synthesis of transitory amylose and amylpectin from ADP-D-glucose in pulse-chase experiments showed that labelled D-glucose decreased in the amylose fraction and appeared in the amylopectin fraction.[38] However, time-course experiments showed that the rate of synthesis of amylopectin was higher than that of amylose at an early stage, suggesting a certain degree of independent synthesis of the two fractions. Both ADP- and UDP-D-glucose have been used as D-glucosyl donors in the measurement of starch synthetase activity in the developing endosperm of barley.[39] An increase in activity could be detected in the amyloplast fraction during maturation of the endosperm. The starch synthetase from grape (*Vitis vinifera*) leaves has been shown to be active both in the presence and in the absence of primer, but the corresponding enzyme from maize (*Zea mays*) leaves and kernels synthesized polyglucan in the absence of primer.[40] A review has discussed the genetic regulation of the synthesis of starch–biosynthetic enzymes in the period during which the synthesis of starch occurs; the allosteric regulation of the biosynthesis of the sugar–nucleotide precursor molecule, ADP-D-glucose, was also discussed.[41] Branching enzyme, *viz.* α-1,4-glucan:α-1,4-glucan 6-glucosyltransferase, has been resolved chromatographically into two fractions which stimulated 'unprimed activity' catalysed by α-glucan synthetase.[42] However, the branching enzyme did not stimulate the 'primed activity'. Stimulation of the 'unprimed' activity by branching enzyme was explained by its catalysis of the formation in the

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growing glucan of an increased number of non-reducing chain-ends, which are able to accept D-glucosyl residues from ADP-D-glucose. The use of ^{13}C-labelled starch granules as a precursor of uniformly labelled D-[^{13}C]glucose has been achieved by growth of potato plants in an atmosphere of ^{13}C-enriched carbon dioxide.[43] The distribution of the ^{13}C-label among the six carbon atoms of the monosaccharide appeared to be nearly uniform.

Improved results in the determination of cell-wall constituents have been obtained by prior digestion of the samples with a commercial amyloglucosidase, which hydrolysed starch without appreciable loss of cell-wall components.[44] The maturation of wheat is characterized by increases in the contents of pentosan and starch of the kernel at the expense of available sugars (total reducing and non-reducing sugars, and mono-, di-, and tri-saccharides).[45] In elongating pea-stem segments, the turnover of cell-wall polysaccharides, and the effects of auxin thereon have been measured.[46] Whilst most wall polymers, including galacturonans and cellulose, did not undergo any appreciable turnover, 20% of the starch was mobilized. In the pectin-extracted material, there was a 50% decrease in the level of D-galactose. No changes were affected by indolyl acetic acid, although it was suggested that auxin induced the transformation of wall xyloglucan from an insoluble form to a water-soluble form. Cell-wall strength was shown to decrease on treatment with auxin and at low pH.[47] The amount of xyloglucan fragments bound to cellulose was sensitive to both temperature and non-aqueous solvents. However, neither the level of xyloglucan bound to cellulose at equilibrium nor the rate at which these molecules bind was sensitive to changes in the hydrogen-ion concentration. The results support the view that xyloglucan chains are connected to cellulose fibres by hydrogen bonds within the cell wall, but the interconnection between the two polysaccharides is unlikely to involve the site within the wall that regulates the rate of cell elongation. The biological significance of the shapes of plant cell walls and of reserve polysaccharides has been reviewed,[48] and a review on Raman spectroscopy of biological molecules has included information on amylose and amylopectin.[48a]

Cellulose

A method has been described for the isolation of cell walls in the starchy endosperm from milled rice and Saké mash.[49] The composition of the walls, which caused aggregation of yeasts, was examined and found to contain cellulose and hemicelluloses. Aggregation was caused by cellulose but could be prevented by heating the cells with anionic or cationic surfactants.[50] Apple cellulose appeared to have either a higher molecular weight or a higher steric orientation than some of the better known celluloses, although apples harvested after a severe winter

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did not yield cellulose with these characteristics. The results were discussed with a view to providing possible explanations of problems of storage and processing encountered with the fruit.[51] Cellulose III_I, cellulose III_{II}, cellulose IV_I, and cellulose IV_{II} have been distinguished from each other by meridional *X*-ray patterns.[52] Despite chain-packing in the unit cell, the four polymers were considered to have different molecular conformations. Chain conformations in the cellulose I family (I, III_I, and IV_I) and in the cellulose II family (II, III_{II}, and IV_{II}) have been shown to differ from each other, with the former having 'bent' and the latter 'bent–twisted' conformations.[53] The mechanism of the transformation of cellulose fibres into cellulose IV has been examined.[54] Heat treatment caused a relaxation of intermolecular hydrogen bonds, resulting in a change to a more symmetrical structure. The relative availability and disposition of the hydroxy-groups at C-2, C-3, and C-6 of the D-glucopyranosyl units of a particular, highly ordered hydrocellulose II have been studied by means of the reaction with *NN*-diethylaziridinium chloride.[55] The orientation behaviour of regenerated cellulose in both crystalline and non-crystalline phases has been investigated during coagulation–regeneration from viscose solution and during drying of the resulting gel film.[56] It was found that the stronger the tensions arising parallel to the surface during coagulation–regeneration and drying of the gel film, the more prominent became the uniplanar orientation of the (101) non-crystalline chain-segments parallel to the surface of the film. These changes were associated with considerable distortion and disintegration of the regenerated crystal. The fractionation of cellulose has been carried out in solutions of cadoxen.[57] It has been suggested that the thermal degradation of cellulose occurs by random nucleation and nucleus growth in the cellulose fibrils so as to yield a carbon whose microporous structure replicates the pore system in the parent cellulose.[58] A thermochemical approach to flame retardation for cellulosic materials has discussed dehydration processes of a number of related carbohydrates from the thermochemical standpoint.[59] The mechanism of degradation of cellulose by radiation has been explained by considering a fibril model, with spread chains for cellulose I and with overlapping, folded chains for cellulose II.[60] The crystallinity of cotton cellulose during heterogeneous hydrolysis with acid increased rapidly; the value calculated from i.r. measurements soon became constant, whereas the value obtained from specific-volume data reached a maximum.[61] It was also found that the hydrolysis caused a shift in the O–H stretching band to a new position of lower frequency, indicating that the hydrolysis of cotton fibres resulted in a strengthening of the hydrogen bonds, whereas

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the reverse was true of mercerization. The transformation of cellulose I into cellulose II was more effective when treatment was carried out with a mixture of sodium hydroxide and sodium sulphide than with sodium hydroxide alone.[62] The degradation of cellulose by the action of nitrogen tetraoxide in DMF seems to be accounted for by both solvation and coagulation.[63] The production of hydrogen peroxide by wood-rotting fungi has led to the suggestion that these organisms may employ a peroxide–Fe^{2+} mechanism to decompose the wood cellulose or to render it more susceptible to attack by conventional cellulases.[64] Cellulose has been converted into oxycellulose, with the production of reducing properties, on exposure to iron powder under conditions of rusting.[65] Direct depolymerization of cellulose is considered to be brought about by free radicals produced by the Fe^{2+} system. In a study of the oxidation of some hydrocelluloses with chromic acid, the rate constant was shown to decrease sharply in the initial stages of the reaction, after which it attained a constant, levelling-off value.[66] The variation with temperature of the rate constants for oxidation of cotton cellulose and viscose rayon with chromic acid has been studied in order to test for the existence of a thermal transition.[67] The accessibility of water to a range of cotton, wood, and acid-hydrolysed celluloses has been investigated by ^1H n.m.r. spectroscopy following exchange with D_2O.[68]

The hydrolysis of alkali-treated cotton with acid released large proportions of 3-deoxy-*ribo*-hexonic, 3-deoxy-*arabino*-hexonic, and 2-*C*-methylglyceric acids, together with a minor proportion of 2-*C*-methylribonic acid.[69] Reduction of the cellulose end-groups and subsequent analysis of the hydrolysate revealed 3-deoxy-*ribo*-hexitol, 3-deoxy-*arabino*-hexitol, 2-*C*-methylglycerol, and 2-*C*-methylribitol. It was concluded from these results that, in addition to 3-deoxyhexonic acid end-groups, significant quantities of terminal 2-*C*-methylglyceric acid groups and minor amounts of 2-*C*-methylribonic acid groups are formed during alkali treatment. Reduction, with sodium borohydride, of cellulose subjected to oxygen–alkali treatment resulted in the formation of D-glucitol, D-mannitol, and D-erythritol end-groups.[70] Cleavage of the cellulose chains by β-elimination formed D-glucose end-groups, which gave rise to terminal D-mannose and D-fructose moieties by isomerization. In addition to the aforementioned polyols, arabinitol, threitol, allitol, and altritol were present. The virtual absence of D-arabinose end-groups after treatment of native cotton with alkali, and their presence after the like treatment of hydrocellulose, supported an earlier assumption that any D-arabinose end-groups formed are rapidly removed by β-elimination. The formation of equal amounts of allitol and D-altritol suggested the presence of 2,3-hexodiulose end-groups as intermediates, which would also explain the formation of end-groups of D-erythrose and D-erythronic acid. The possibility that the reducing

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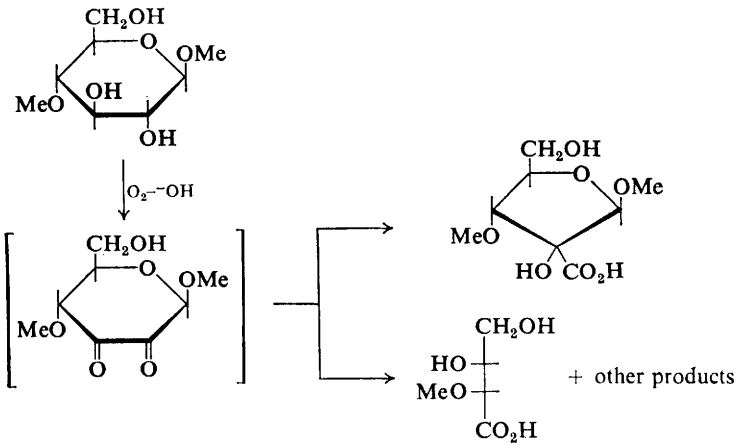
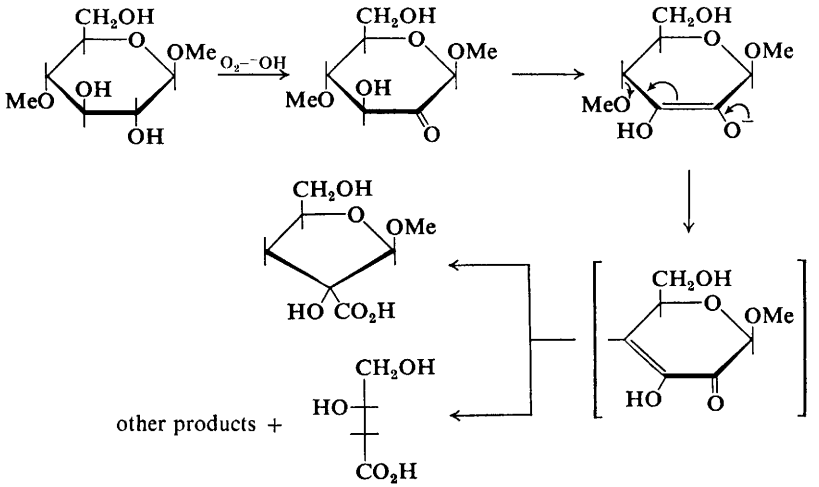
end-groups of cellulose are oxidized primarily to D-glucosone end-groups was evaluated. D-Glucosone on oxygen–alkali degradation was found to give D-arabinonic acid and D-erythronic acid, indicating that degradation proceeds, in part, *via* the D-glucosone pathway.[71] During oxygen-bleaching of cellulose, iron and cobalt compounds were found to favour the formation of carbonyl groups along the chains.[72] The reactions giving rise to carbonyl groups were catalysed more effectively than those leading to their destruction, whilst the degradation of hydrocellulose was slightly retarded during oxygen–alkali treatment in the presence of vanadate compounds. A study has been made of the degradation of 4-*O*-methyl-D-glucose in sodium hydroxide solution, with and without the presence of small amounts of calcium ions, in an attempt to simulate the alkali-peeling reaction of cellulose.[73] The main degradation products observed were α- and β-glucoisosaccharinic acids, indicative of peeling reactions, but α- and β-4-*O*-methylglucometasaccharinic acids, which would be expected to arise from the stopping reaction, were not detected. In the presence of small amounts of iron compounds, the oxidation of cellobiitol was effectively retarded by the addition of magnesium and lanthanum salts, due to radical scavenging by the transition-metal ions.[74] An excess of magnesium hydroxide was found to exert additional protection and tended to increase the level of peroxide formed, whereas an excess of lanthanum hydroxide had the opposite effects. The rate of attack upon cellobiitol by oxygenated alkali increased with increasing concentrations of copper and iron salts, as it did with cobalt salts, but in the latter case the highest rate of reaction was obtained at low concentrations.[75] This effect was ascribed to the fact that precipitated cobalt(III) hydroxide acted as an inhibitor. The major products from the oxidation of methyl 4-*O*-methyl-β-D-glucopyranoside with oxygen in alkali were formic, acetic, and glycolic acids, methanol, and ethanol.[76] The most significant reaction products were methyl 2-*C*-carboxy-3-*O*-methyl-β-D-pentofuranosides, methyl 2-*C*-carboxy-3-deoxy-β-D-pentofuranosides, and tetronic acids, which were probably formed by rearrangement of the corresponding 2,3-diketonic and 4-deoxy-2,3-diketonic intermediates, as illustrated in Schemes 1 and 2. Oxidation of methyl β-D-glucopyranoside with either oxygen or hydrogen peroxide in alkali gave similar degradation products, but in different proportions.[77] The major products obtained in the oxidation with oxygen were the acids (1) and (2), whereas the major product obtained with hydrogen peroxide was the isomeric acid (3).

D-Gluconic acid was found to be the most abundant terminal carboxylic acid formed during the chlorination of cellulose and hydrocellulose, together with minor amounts of terminal D-arabinonic and D-erythronic acids.[78] Large amounts of D-glucuronic and cellobiouronic acids were detected after hydrolysis, demonstrating that oxidation at C-6 along the cellulose chains was significant.

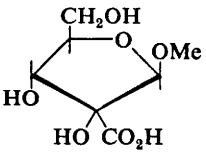
[71] B. Ericsson, B. O. Lindgren, and O. Theander, *Cellulose Chem. Technol.*, 1973, **7**, 581.
[72] M. Manoucheri and O. Samuelson, *Svensk Papperstidn.*, 1973, **76**, 486.
[73] M. Stén and T. Mustola, *Cellulose Chem. Technol.*, 1973, **7**, 359.
[74] O. Samuelson and L. Stolpe, *Svensk Papperstidn.*, 1974, **77**, 16.
[75] O. Samuelson and L. Stolpe, *Svensk Papperstidn.*, 1974, **77**, 513.
[76] B. Ericsson and R. Malinen, *Cellulose Chem. Technol.*, 1974, **8**, 327.
[77] B. Ericsson, B. Lindgren, and O. Theander, *Cellulose Chem. Technol.*, 1974, **8**, 363.
[78] B. Alfredsson and O. Samuelson, *Svensk Papperstidn.*, 1974, **77**, 449.



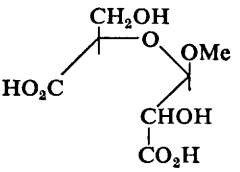
Scheme 1



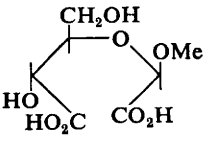
Scheme 2



(1)



(2)



(3)

Appreciable quantities of 3-deoxypentonic acids and 2-deoxy-*erythro*-pentonic acid were also detected in the hydrolysates, even after the cellulose had been reduced with sodium borohydride before hydrolysis, indicating that the former acids are derived from non-terminal hexosulose moieties. Sensitized photodegradation of cellulose and cellulose waste has been achieved by using anthraquinone 2-sulphonate and proflavin dihydrochloride, producing materials similar in appearance to enzyme-degraded celluloses.[79] The different responses of cellulosic substrates to cellulases produced by *Penicillium funiculosum* have been attributed to variations in particle size, surface area, crystallinity, and dimensions of the crystallites.[80] *X*-Ray diffraction revealed that the crystallinity increased by 7—10% as a result of enzymic hydrolysis, with no appreciable change in the dimensions of the crystallites; this indicates that enzymic action is confined primarily to paracrystalline regions of the surface. A purified, cellulolytic C_1 component isolated from *Trichoderma koningii* released terminal cellobiose units from cellulose, the extent of its action being determined principally by the product and by the nature of the substrate.[81] The relation of enzyme-catalysed β-(1 → 4)-glucan synthesis to cellulose synthesis in plants has been reviewed.[82] The distribution, isolation, composition, and structure of cellulose have been discussed in a treatise on the surface carbohydrates of eukaryotic cells.[83]

Gums, Mucilages, and Pectic Substances

The limitations of a method for the identification of commercial, hydrocolloid stabilizing agents[84] have been overcome by one that facilitated the positive identification of pectinate and gum tragacanth and of guar gum and locust gum in mixtures.[85] The results of studies on the compositions and properties of gum exudates from subspecies of *Acacia tortilis* have proved to be of chemotaxonomic interest.[86] The compositions and solution properties of Indian and Papuan specimens of the gum of *Anacardium occidentale* have been shown to be closely similar.[87] Contrary to earlier reports, the gum does not contain D-galacturonic acid, but it does contain D-glucose. The molecular weight of the gum increased on storage, probably by self-association, with the formation of a small proportion of a component of very high molecular weight. In an attempt to find the dissociation constants of gum arabic and polyacrylic acids, an equation has been derived to account for the 'Pallman or sol-concentration effect'.[88] Acetone-precipitated neem (*Azadirachta indica*) gum contains both carbohydrate and protein which, although inseparable by gel-permeation chromatography, can be separated into a number of components having different ratios of carbohydrate and protein by TEAE–cellulose chromatography.[89] Eucalyptus gum, after

[79] K. Eskins, B. L. Bucher, and J. H. Slonecker, *Photochem. Photobiol.*, 1973, **18**, 195.
[80] S. M. Betrabet, V. G. Khandeparkar, and N. B. Patil, *Cellulose Chem. Technol.*, 1974, **8**, 339.
[81] G. Halliwell and M. Griffin, *Biochem. J.*, 1973, **135**, 587.
[82] C. L. Villemez, in ref. 1, p. 183.
[83] G. M. W. Cook and R. W. Stoddart, 'Surface Carbohydrates of the Eukaryotic Cell', Academic Press, London and New York, 1974, p. 165.
[84] R. G. Morley, G. O. Phillips, D. M. Power, and R. E. Morgan, *Analyst*, 1972, **97**, 315.
[85] R. G. Morley, G. O. Phillips, D. M. Power, and R. E. Morgan, *Analyst*, 1973, **98**, 813.
[86] D. M. W. Anderson and P. C. Bell, *Phytochemistry*, 1974, **13**, 1875.
[87] D. M. W. Anderson, P. C. Bell, and J. R. A. Millar, *Phytochemistry*, 1974, **13**, 2189.
[88] B. N. Gosh, *J. Indian Chem. Soc.*, 1974, **51**, 57.
[89] V. S. Narayan and T. N. Pattabiraman, *Indian J. Biochem. Biophys.*, 1973, **10**, 155.

isolation in alkali medium, contained D-xylose and 4-*O*-methyl-D-glucuronic acid.[90] Acidic hydrolysis of the methylated polysaccharide furnished 2,3-di-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose, and 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylose (221 : 1 : 25). Coupled with optical rotational data and the results of periodate oxidation, the structure assigned (4) was that of a (1 → 4)-β-D-glycan containing one 4-*O*-methyl-D-glucopyranosyluronic acid unit attached to every tenth D-xylopyranose unit by a (1 → 2)-linkage.

4-*O*-MeGlcUA*p*
1
↓
2
[→ 4-β-D-Xyl*p*-1-]$_4$ → 4-β-D-Xyl*p*-1 → [4-β-D-Xyl*p*-1-]$_5$
(4)

Gum exudates from *Terminalia sericea* and *T. superba* contain D-glucuronic acid, D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid, as well as D-galactose, L-arabinose, L-rhamnose, D-mannose, and D-xylose.[91] Although both exudates have similar compositions with respect to the proportions of neutral sugars and total uronic acids, that of *T. sericea* contains more 4-*O*-methyl-D-glucuronic acid.

A mucilage isolated from the tubers of *Bletilla striata* has been identified as a glucomannan of molecular weight 1.8×10^5.[92] Partial acidic hydrolysis and periodate oxidation suggested the existence of β-(1 → 4)-linked aldohexopyranose units containing, on average, six aldopentose units per end group. Odoratan and falcatan, mucilages isolated from the rhizomes of *Polygonatum odoratum* and *P. falcatum*, respectively, released six oligosaccharides (5)—(10) on partial hydrolysis with acid.[93]

β-D-Glc*p*-(1 → 4)-D-Man
(5)

β-D-Man*p*-(1 → 4)-D-Man
(6)

β-D-Man*p*-(1 → 4)-D-Glc
(7)

β-D-Man*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-D-Man
(8)

β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-D-Man
(9)

β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-D-Glc
(10)

[90] S. P. Singh, R. D. Guha, and J. S. Negi, *Indian J. Chem.*, 1973, **11**, 876.
[91] D. M. W. Anderson and P. C. Bell, *Phytochemistry*, 1974, **13**, 1871.
[92] M. Tomoda, S. Nakatsuka, M. Tamai, and M. Nagata, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2667.
[93] M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2511.

Direct evidence for a supramolecular structure of pectate has been presented.[94] Both positive and negative staining have indicated that these tertiary structures exist as elementary fibrils, possibly as a means of stabilizing the component molecules of a gel network, thus explaining differences in molecular weights determined by end-group analysis compared with other methods. Cations of calcium, magnesium, and potassium and anions of citrate, malate, phytate, and chloride have been shown to stimulate the β-eliminative breakdown of esterified pectin.[95] The association of the cations with the pectin polyanion decreased the overall negative charge and facilitated the approach of the hydroxy-ion necessary for the initiation of β-elimination. By use of a continuous-flow procedure over an immobilized polygalacturonase, oligomeric breakdown products have been isolated from a polygalacturonate.[96] The distribution, structure, and histology of pectins have been reviewed.[83]

The pectin of *Matricaria chamomilla* has been found to contain D-galacturonic acid, D-galactose, D-glucose, L-rhamnose, L-arabinose, and D-xylose.[97] Pectic polysaccharides extracted from the cotyledons of kidney beans have been fractionated into neutral polysaccharides and weakly acidic pectinic acids, some of which contained polygalacturonase-resistant D-galacturonic acid residues partly esterified by methyl groups.[98] The results of extraction of hydroxyproline-containing proteins and pectic substances from the cell walls of growing and resting hypocotyl segments of *Phaseolus aureus* indicated a cell-wall structure of this tissue in which at least part of the pectin is not linked covalently to either glycoprotein or other cell-wall constituents.[99] In elongating pea-stem segments, the principal process in the turnover of wall polymers involved a 50% decrease in D-galactose in the pectinase-extractable fraction, which included D-galactose attached to a pectinase-resistant rhamnogalacturonan.[46] The patterns of incorporation of radioactivity from D-[*U*-^{14}C]glucose into the pectic components of sections of sycamore roots and into maize root-cap slime have been compared.[100] The slime is considered to be a form of pectin, modified so as to provide a hydrated protective coating around the root-tip. Sugars supplied to germinating seedlings of *Zea mays* regulated the secretion of polysaccharides by the outer cells of the root-cap.[101] The secreted polysaccharides contained D-glucose, D-galactose, and D-galacturonic acid, and smaller proportions of D-mannose, L-arabinose, D-xylose, L-fucose, and L-rhamnose. A pectin isolated from the hulls of rape seed was shown to have a degree of esterification of 83% and to contain residues of D-galacturonic acid, D-galactose, L-arabinose, D-xylose, L-rhamnose, and L-fucose.[102] Partial acidic hydrolysis of the derived pectic acid furnished 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid, the polymer-homologous tri- and tetra- saccharides,

[94] G. F. Leeper, *J. Texture Studies*, 1973, **4**, 248.
[95] M. J. H. Keijberts and W. Pilnik, *Carbohydrate Res.*, 1974, **33**, 359.
[96] F. E. A. van Houdenhoven, P. J. G. M. De Wit, and J. Visser, *Carbohydrate Res.*, 1974, **34**, 233.
[97] A. G. Gorin and A. I. Yakovlev, *Khim. prirod. Soedinenii*, 1974, 137.
[98] Y. Matsura, C. Hatanaka, and J. Ozawa, *J. Agric. Chem. Soc. Japan*, 1973, **47**, 497.
[99] R. W. Bailey and H. Kauss, *Planta*, 1974, **119**, 233.
[100] K. Wright and D. H. Northcote, *Biochem. J.*, 1974, **139**, 525.
[101] D. D. Jones and D. J. Morré, *Physiol. Plant.*, 1973, **29**, 68.
[102] G. O. Aspinall and K. S. Jiang, *Carbohydrate Res.*, 1974, **38**, 247.

and 4-*O*-(D-glucopyranosyluronic acid)-L-fucose. A close similarity between this pectin and lemon-peel pectin was revealed by the similarity of the cleavage products from the methylated pectins on examination by g.l.c.–m.s. The water-soluble pectic polysaccharides from four strains of *Rosa glauca* have been examined in relation to their morphological and histological characteristics.[103] D-Galactose and D-galacturonic acid were identified as the main components, with lesser amounts of L-arabinose, L-rhamnose, D-glucose, D-xylose, and L-fucose also present. In the presence of high concentrations of unlabelled *myo*-inositol, the incorporation of D-[6-^{14}C]glucose into the cell-wall D-galacturonic acid of the roots of *Zea mays* was significantly reduced, although that into the cell-wall D-glucosyl units remained unchanged.[104] It was inferred that, in order to be converted into the uronic acid, D-glucose must first pass through the internal *myo*-inositol pool of the roots.

Hemicelluloses

Some of the factors influencing the separation of xylan and galactoglucomannan-type wood hemicelluloses have been discussed.[105] The importance of delignification procedures was demonstrated, and precipitation of galactoglucomannans with barium hydroxide has yielded a fraction highly enriched in xylan. As a solvent for cell-wall polysaccharides, DMSO has been shown to give higher yields of water-soluble components than either aqueous or alkaline extracts.[106] The addition of iodide ion has given increased stabilization to the polysaccharides in the extraction of wood pulp with oxygenated alkali.[107] Twenty species of leaves have been shown to contain polymer-bound hexosamines in similar amounts.[108] It was suggested that the amino-sugars may be an essential component of the membrane structure. A polysaccharide fraction containing D-galactose, D-glucose, D-mannose, small amounts of amino-sugars, and appreciable quantities of uronic acids, and sulphate and acetyl groups exhibited hypolipidaemic activity in rats fed on a high-fat, high-cholesterol diet.[109] Both cellulose and hemicelluloses from *Heteropogon contortus* were incompletely digested in bovine rumen; the resistance was probably due to physical protection of the polysaccharides by lignin.[110] In *Hordeum vulgare* plants at different stages of maturity, the proportion of D-xylose residues in the total hemicelluloses increased as the tissues matured, that of D-galactose varied little, whereas the proportions of L-arabinose, D-glucose, and D-glucuronic acid decreased.[111] As well as a decrease in $\overline{DP}_n$ of the β-glucans, there is also a decrease in the ratio of β-(1 → 3)- to β-(1 → 4)-linkages with maturity. Hulls of sweet and bitter lupin (*Lupinus angustifolius*) seeds have been shown to contain pectin, cellulose, and hemicellulose;[112] the alkali-soluble fraction was shown to consist largely of xylan, but some mannan

[103] A. Mollard, G. Hustache, and F. Barnoud, *Physiol. Veg.*, 1973, **11**, 539.
[104] R. M. Roberts and F. Loewus, *Plant Physiol.*, 1973, **52**, 646.
[105] G. G. S. Dutton, B. I. Joseleau, and P. E. Reid, *Tappi*, 1973, **56**, 168.
[106] E. Maekawa, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 75.
[107] J. L. Minor and N. Sanyer, *Tappi*, 1974, **57**, 109.
[108] D. Racusen and M. Foote, *Canad. J. Bot.*, 1974, **52**, 2111.
[109] K. S. Devi and P. A. Kurup, *Atherosclerosis*, 1973, **18**, 389.
[110] R. F. H. Dekker and G. N. Richards, *Carbohydrate Res.*, 1973, **27**, 1.
[111] A. J. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1974, **13**, 1347.
[112] R. W. Bailey, S. E. Mills, and E. L. Hove, *J. Sci. Food Agric.*, 1974, **25**, 955.

was also present. The coexistence of the molecular-weight distribution and the chemical inhomogeneity of the reed hemicelluloses isolated from *Phragmites communis* and *Arundo donax* has led to uncertain results in a study of the polydispersity of the polysaccharides by classical methods.[113] Studies on the polydispersity of the hemicellulose derivatives did not afford any information about the initial product distribution. The wood of *Pinus radiata* has been shown to contain 70% total polysaccharides, made up of cellulose, acetylated galactoglucomannan, and arabino-4-*O*-methylglucuronoxylan.[114] The inner-bark carbohydrates of *Pseudotsuga menziesii* have been fractionated into a xylan, a galactoglucomannan, a glucomannan, and a glucan-rich residue.[115] A branched xylan from rye grass was found to maintain a similar composition throughout the growth of the plant and to increase in concentration during growth.[116] The major polysaccharide of this species is a linear xylan, which exhibited considerable polydispersity in that the ratio of D-xylose to L-arabinose rose markedly during growth, but its concentration in the plant tissue, as a proportion of the total hemicelluloses, slowly declined during growth. After digestion of wheat-straw hemicellulose B with hemicellulase, the isolated product (molecular weight 5—10 × 10^3) still contained carcinostatic activity.[117] Under the influence of 2-chloroethyltrimethylammonium chloride, no variation was found in the relation between the rates of [^{14}C]-incorporation by different cell-wall fractions.[118] Cell-elongation was inhibited to a greater extent than the synthesis of these polysaccharides. It has been proposed that the regulation of cell-wall extension could be effected by cell-wall lectins functioning in the form of a non-covalent 'glueing' substance, possibly through covalent linkages to certain polysaccharides and simultaneous formation of a connection to other polysaccharides possessing the lectin-specific sugar groups.[119] Cell walls from sections of elongating and non-elongating lupin hypocotyls yielded an arabinoxylan containing hydroxyproline residues after sequential extraction.[120] The polymer isolated from non-elongating walls had a much higher proportion of L-arabinosyl and D-galactosyl residues than the corresponding polymer recovered from elongating walls. The results suggested the existence of a hydroxyproline-containing glycoprotein within the wall of non-elongating tissue, consistent with its proposed role in arresting the elongation of cells. Indolyl acetic acid promoted the liberation of a water-soluble xyloglucan from stem segments of *Pisum sativum*.[121] The xyloglucan effect of auxin occurred normally when elongation was blocked by D-mannitol. The amounts and rates of export of polysaccharides occurring within the membrane system of maize-root cells, after incubation *in vivo* with D-[*U*-^{14}C]glucose, have been measured.[122] After the membrane compartments had reached a saturation value of radioactivity in polysaccharide components,

[113] C. V. Uglea, *Makromol. Chem.*, 1974, **175**, 1535.
[114] J. A. Smelstorius, *Holzforschung*, 1974, **28**, 67.
[115] M. L. Laver, C. H. Chen, J. V. Zerrudo, and Y. C. L. Lai, *Phytochemistry*, 1974, **13**, 1891.
[116] I. M. Morrison, *Carbohydrate Res.*, 1974, **36**, 45.
[117] Y. Sugihara and F. Araki, *Hiroshima J. Med. Sci.*, 1973, **22**, 29.
[118] W. Bleiss, E. Paul, and H. Göring, *Biochem. Physiol. Pflanzen*, 1973, **164**, 414.
[119] H. Kauss and C. Glaser, *F.E.B.S. Letters*, 1974, **45**, 304.
[120] J. A. Monro, R. W. Bailey, and D. Penny, *Phytochemistry*, 1974, **13**, 375.
[121] J. M. Labavitch and P. S. Ray, *Plant Physiol.*, 1974, **54**, 499.
[122] D. J. Bowles and D. H. Northcote, *Biochem. J.*, 1974, **142**, 139.

the radioactivity in exported polysaccharides continued to increase. The latter were formed and maintained by a steady-state turnover of synthesis and transport from the membrane system. The walls of six suspension-cultured monocotyledons have been shown to contain cellulose, uronic acids, and protein; an arabinoxylan was identified as the major component of the primary cell walls, and a mixed linkage β-D-glucan was found in only one of the cell-wall preparations.[123]

An arabinan, investigated by classical structural methods, has been found to consist of a highly branched structure having an average repeating unit of 27 sugar residues, including 11 terminal, non-reducing L-arabinosyl units.[124] Branching was shown to occur through positions 2, 3, and 5. Physicochemical differences have been demonstrated in two reed xylans from *Arundo donax* and *Phragmites communis*.[125] The viscous product from rye, isolated during extraction of proteins, has been separated by ion-exchange chromatography into a number of glycopeptides having backbones of β-D-xylopyranose units carrying α-L-arabinofuranosyl branches.[126] Using high concentrations of UDP-D-xylose and cell-free preparations of xylan synthetase from *Avena sativa*, three polysaccharides (other than a neutral xylan) were synthesized, two being glucuronoxylans and one, possibly, a glycolipid.[127] The enzyme was stimulated *in vivo* by pretreatment with the growth inhibitor peroxyacetyl nitrate, although inhibition resulted on incubation of the enzyme and the inhibitor *in vitro*.[128] It was concluded that the synthetase was protected internally from the growth inhibitor.

A method has been described for the estimation of β-glucan in barley extracts involving a differential assay; the D-glucose content of a total acid hydrolysate is determined by glucose oxidase, and the α-glucan content is determined after treatment of the extract with amyloglucosidase.[129] The difference between these determinations represents D-glucose derived from the β-glucan. Although β-glucans isolated from ungerminated and malted barley contained the same proportion (3 : 1) of β-(1 → 4)- to β-(1 → 3)-linkages, the two polysaccharides were not hydrolysed to the same extent either by a partially purified, bacterial *endo*-β-1,3-glucanase or by a homogeneous *endo*-β-1,3-glucanase from malted barley.[130] Studies of viscosity and reducing-power have led to the suggestion that the overall arrangement of linkages, and, hence, the susceptibility to enzymic attack, differs according to the source and the method of extraction of the glucans. A hemicellulosic β-glucan isolated from the hypocotyls of *Phaseolus aureus* was found to contain (1 → 3)- and (1 → 4)-linked D-glucopyranosyl residues (1.0 : 1.7).[131] Oligosaccharides containing both β-(1 → 3)- and β-(1 → 4)-linked residues were isolated from partial acidic hydrolysates of the β-glucan. Gluco- and galacto-lipids have been isolated from plasmalemma-rich fractions of cell homogenates of bean seedlings.[132] Incubation of [^{14}C]-labelled plasmalemma

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[124] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1974, **36**, 35.
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[127] R. Ben-Arie, L. Ordin, and J. I. Kindinger, *Plant and Cell Physiol.*, 1973, **14**, 434.
[128] R. Ben-Arie, L. Ordin, and J. I. Kindinger, *Plant and Cell Physiol.*, 1973, **14**, 435.
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[131] A. J. Buchala and G. Franz, *Phytochemistry*, 1974, **13**, 1887.
[132] G. Franz, *Verhandl. Schweiz. Naturforsch. Ges.*, 1972, **152**, 148.

material with unlabelled nucleotide diphosphate D-glucoses showed partial transfer of the label from the D-glucose–lipid moiety to a polysaccharide; the lipid appeared to function as a transfer substance only in its membrane-bound form. A particulate enzyme from *Lupinus albus*, which is responsible for the synthesis of β-(1 → 4)-D-glucan from UDP-D-[^{14}C]glucose, has been isolated and some of its properties established.[133] Enhancement of β-(1 → 4)-glucan synthesis and inhibition of β-(1 → 3)-glucan synthesis were obtained in the presence of magnesium ions. The specific activity of D-[U-^{14}C]glucose incorporated into lipid, starch, and cell-wall fractions of *Lolium multiflorum* has been measured in endosperm cultures during pulse-chase experiments.[134] The starch fraction initially showed a high specific activity, which declined during the chase period, whereas the wall fraction (mainly mixed-linkage β-glucans) remained fairly constant throughout. Variation of the concentration of UDP-D-glucose or of the proportions of plasma membrane or dictyosomes of onion (*Allium cepa*) stem was followed by the synthesis of β-(1 → 3)- and β-(1 → 4)-glucans in different proportions and at different rates.[135] The capacity to synthesize glucans resided in both the Golgi apparatus and the plasma membrane, but the plasma membrane had the greater capacity for the synthesis of alkali-insoluble glucans at high concentrations of UDP-D-glucose. The primary leaves of *Phaseolus vulgaris* showed a decreased synthesis of lipid and β-glucan after heat shock, although recovery of this inhibition was achieved after a period of time.[136] Synthesis of an alkali-insoluble glucan by cell-free preparations was stimulated, both in absolute and relative terms, in treated leaves at the later time period. Treatment of pea-stem segments with indolyl acetic acid caused an increase in activity of a particulate, UDP-D-glucose-dependent, β-glucan synthetase activity.[137] The effect was also demonstrated with the polysaccharide synthetase activity using either UDP-D-galactose or UDP-D-xylose as substrates, and, to a lesser extent, with a GDP-D-glucose-dependent, glucan synthetase activity. When soluble pea enzyme was supplied with sucrose and UDP, and the preparation was then supplemented with particles possessing β-glucan synthetase activity, the D-glucose moiety of sucrose was converted into glucan *in vitro*.[138] The results indicated that it is feasible for the synthetases to co-operate *in vivo* to generate β-glucan for expanding cells.

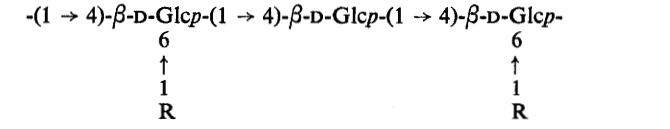
An electrophoretically homogeneous glucomannan has been isolated from the tubers of *Arum orientale* by adsorption on to glutaraldehyde-insolubilized concanavalin A.[139] The presence of β-D-glucosyl and β-D-mannosyl residues in (1 → 4)-linkage was inferred from i.r. and periodate-oxidation studies. Evidence for the existence of acetyl groups in a glucomannan isolated from *Lilium longiflorum* was obtained from i.r. and ^{1}H n.m.r. spectroscopy.[140] A polysaccharide

[133] G. L. Larsen and D. O. Brummond, *Phytochemistry*, 1974, **13**, 361.
[134] R. L. Anderson and B. A. Stone, *Proc. Austral. Biochem. Soc.*, 1974, **7**, 33.
[135] W. J. Van Der Woode, C. A. Lembi, D. J. Morré, J. I. Kindinger, and L. Ordin, *Plant Physiol.*, 1974, **54**, 333.
[136] L. Ordin, C. Itai, A. Benzioni, C. Musolan, and J. I. Kindinger, *Plant Physiol.*, 1974, **53**, 118.
[137] P. M. Ray, *Plant Physiol.*, 1973, **51**, 601.
[138] J. Rollit and G. A. Maclachlan, *Phytochemistry*, 1974, **13**, 367.
[139] M. I. Koleva and C. Achtardjieff, *Carbohydrate Res.*, 1973, **31**, 142.
[140] T. Matsuo and T. Mizuno, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 465.

($\overline{DP}_n$ 665) containing D-glucose and D-mannose (1 : 3.3) was isolated from the tubers of *Orchis morio*.[141] From structural studies it was concluded that the polysaccharide contains a backbone of β-(1 → 4)-D-glucosyl and -D-mannosyl residues with about seven branch points (probably at O-3) per molecule. Acetyl groups were found to be almost exclusively linked to O-2 or O-3 of the D-mannosyl residues. The polysaccharide appears to be similar to material from the mucilage globules, which were mechanically separated and isolated from the tissue of young tubers. A radioactive glucomannan, prepared using enzymes from *Phaseolus aureus*, has been acetylated and subjected to gel-permeation chromatography.[142] The chains of [^{14}C]glucomannan were not uniformly dispersed, but were separated into two major fractions which may be collections of incompletely resolved polysaccharide chains; the largest fragment had a minimum molecular weight of 2×10^5. Preliminary results suggested that the components of lower molecular weight could be precursors of the components of high molecular weight. Hot-water extraction of *Cassia occidentalis* seeds liberated a galactomannan [D-galactose : D-mannose (1 : 3)] which, after methylation analysis and acidic hydrolysis, yielded 2,3,4,6-tetra-*O*-methyl-D-galactose (1 part), 2,3,6-tri-*O*-methyl-D-mannose (2.2 parts), and 2,3-di-*O*-methyl-D-mannose (1 part).[143] Coupled with the results of periodate oxidation and Smith degradation, the polysaccharide appeared to contain a (1 → 4)-linked backbone to which D-galactosyl residues are attached at O-6. The seeds of eight *Sophora* sp. have been examined for the presence of galactomannan and other water-soluble polysaccharides.[144] Only one species contains a galactomannan, and all but one contain water-soluble polysaccharides composed of D-galactosyl and L-arabinosyl residues. Germinating seeds of lucerne, guar, carob, and soybean initially depleted the raffinose series of oligosaccharides rather than galactomannan.[145] This depletion was accompanied by a rapid increase, followed by a decrease, in the level of α-galactosidase. Methylation, periodate oxidation, and Smith degradation have shown that the arabinogalactan isolated from bamboo shoots contains a backbone of β-(1 → 3)-linked D-galactopyranosyl residues to which L-arabinofuranosyl residues are attached at O-6.[146] A polysaccharide composed of L-arabinose and D-galactose (1 : 3) has been isolated from the pods of *Opuntia dillenii*.[147] On the basis of periodate-oxidation and methylation data, it was postulated that the polysaccharide is composed of interior chains of β-(1 → 4)-linked D-galactopyranosyl units to which non-reducing L-arabinofuranosyl residues are linked at O-3. A small number of D-galactopyranosyl side-chains are also present, attached to O-3 of the main chain. An alkali-soluble polysaccharide from the leaf- and stem-tissues of red clover (*Trifolium pratense*) has been found to contain D-galactose, D-glucose, and D-mannose (1 : 4 : 4.4).[148] A structure consisting of a main chain of β-(1 → 4)-linked D-glucopyranosyl and D-mannopyranosyl residues, to which are attached α-(1 → 6)-linked D-galactopyranosyl residues, was proposed.

[141] A. J. Buchala, G. Franz, and H. Meier, *Phytochemistry*, 1974, **13**, 163.
[142] C. L. Villmez, *Arch. Biochem. Biophys.*, 1974, **165**, 407.
[143] D. S. Gupta and S. Mukherjee, *Indian J. Chem.*, 1973, **11**, 1134.
[144] R. W. Bailey, *New Zealand J. Botany*, 1974, **12**, 131.
[145] B. V. McCleary and N. K. Matheson, *Phytochemistry*, 1974, **13**, 1747.
[146] E. Maekawa and K. Kitao, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 227.
[147] B. K. Srivastava and C. S. Pande, *Planta Medica*, 1974, **25**, 92.
[148] A. J. Buchala and H. Meier, *Carbohydrate Res.*, 1973, **31**, 87.

Amyloids isolated from nasturtium (*Tropaeolum majus*) and tamarind (*Tamarindus indica*) seeds are composed of D-glucose, D-galactose, and D-xylose.[149] From the results of methylation, partial acidic hydrolysis, and enzymic studies, the structure (11) was proposed.

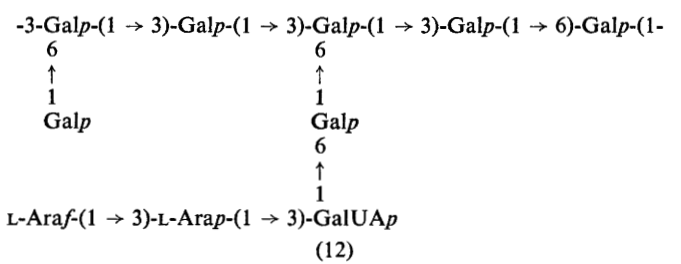


R = α-D-Xyl*p* or -β-D-Gal*p*-(1 → 2)-α-D-Xyl*p* (equal amounts)

(11)

The amyloid associated with the seeds of *Impatiens balsamina* was found to be associated with more protein than the corresponding polymers isolated from nasturtium and tamarind.[150] A polydisperse, neutral polysaccharide (molecular weight 5×10^4) containing D-glucose, D-galactose, D-mannose, and D-xylose has been isolated from a woody peat soil.[151]

The results of methylation, periodate oxidation, and Smith degradation have led to the conclusions that the water-soluble polysaccharide of cashew-nut (*Anacardium occidentale*) shell has a branched structure (12), with a repeating



(12)

unit consisting of a main chain of D-galactosyl units, joined by (1 → 3)- and (1 → 6)-linkages, to which are attached side-chains of D-galactose and an aldobiouronic acid.[152] The aldobiouronic-acid fragment was found to be further substituted at C-3 by an L-arabinosyl side-chain of the type L-Ara*f*-(1 → 3)-L-Ara*p*. The L-arabinosyl side-chains were readily removed by mild hydrolysis with acid, and further hydrolysis of the degraded polysaccharide with acid released the aldobiouronic acid, which was characterized as 6-*O*-(β-D-galactopyranosyluronic acid)-D-galactose.[153] An arabino-4-*O*-methyl-D-glucuronoxylan has been identified as the major hemicellulosic material present in the youngest internodes of the reed *Arundo donax*; it was shown to have very similar structural features to the polysaccharide of mature tissues in being composed of β-(1 → 4)-linked D-xylosyl units.[154] The monocarboxylic acids formed by isomerization of

[149] J. E. Courtois and P. Le Dizet, *Compt. rend.*, 1973, **277**, *D*, 1957.
[150] J. E. Courtois and P. Le Dizet, *Compt. rend.*, 1974, **278**, *C*, 81.
[151] U. Mingelgrin and J. E. Dawson, *Soil Sci.*, 1973, **116**, 36.
[152] S. Bose and P. L. Soni, *Indian J. Chem.*, 1973, **11**, 996.
[153] S. Bose and P. L. Soni, *Indian J. Chem.*, 1974, **12**, 680.
[154] J. P. Joseleau and F. Barnoud, *Phytochemistry*, 1974, **13**, 1155.

aqueous solutions of 4-*O*-methyl-D-glucuronic acid (prepared from the wood of *Betula verrucosa*) have been separated by anion-exchange chromatography and identified by g.l.c.–m.s. as 3-*O*-methyl-D-*lyxo*-5-hexulosonic acid, 3-*O*-methyl-L-*ribo*-5-hexulosonic acid, 4-*O*-methyl-D-mannuronic acid, and 3-*O*-methyl-L-*ribo*-4-hexulosonic acid.[155] The appearance of these acids was explained in terms of de Bruyn–van Eckenstein transformations in which the substituents at C-1, C-2, and C-3 are involved. Two hemicelluloses isolated from *Canavalia ensiformis* and *Triticum aestivum* straws both contain uronic acid, D-galactose, D-glucose, D-xylose, and L-arabinose. Partial hydrolysis with acid and Smith degradation pointed to the existence of similar structural features.[156] The mature tissues of the tropical grass *Panicum maximum* contain an arabino-4-*O*-methyl-D-glucuronoxylan with a main chain comprised of about 46 β-(1 $\rightarrow$ 4)-linked D-xylosyl residues to which are attached seven L-arabinofuranosyl and two 4-*O*-methyl-D-glucopyranosyluronic acid residues, linked through O-3 and O-2, respectively, and a highly branched, acidic arabinogalactoxylan.[157] A hemicellulose of slash pine (*Pinus elliottii*) has been shown by methylation analysis to have a β-(1 $\rightarrow$ 4)-D-xylan chain with many branch points; residues of 4-*O*-methyl-D-glucopyranosyluronic acid, L-arabinofuranose, and D-xylopyranose occur as non-reducing end groups.[158] After *in situ* reduction of extracts of aspen (*Populus tremuloides*) wood, a 4-*O*-methyl-D-glucuronoxylan was isolated in which a few of the uronic acid residues were reduced.[159] The possibility of the presence of an ester- or a lactone-form of some of the 4-*O*-methyl-D-glucuronic acid units in the wood was discussed. Smith degradation and methylation studies on an acidic polysaccharide from soy sauce led to the postulation of a ramified structure resembling that of an acidic polysaccharide in soybean cotyledons.[160] Gel-permeation chromatography of oligosaccharides isolated from soy sauce was monitored by an automated analysis, which indicated a separation of mono-, di-, tri-, tetra-, and poly-saccharides.[161]

The structures of two similar neutral and acidic tetrasaccharides have been established following enzymic hydrolysis of a 4-*O*-methyl-D-glucurono-D-xylan from aspen.[162] It was postulated that a high proportion of 4-*O*-methyl-D-glucuronic acid residues of the xylan could exist in the wood in an esterified form and play an important role in the binding to lignin. The existence of labile uronic acid units in acidic wood hemicelluloses has been reported;[163] these units appear to be moderately resistant to acid but labile to alkali, possibly due to ether and glycosidic linkages to lignin, and to be ester-linked to the xylan chain of the glucuronoxylan. Interpretation of proton signals in the ^{1}H n.m.r. spectra of lignin, carbohydrate, and lignin–carbohydrate model complexes has indicated that 20% of the carbohydrate is lost on hydrolysis.[164] The structure of a homo-

[155] K. Larsson and O. Samuelson, *Carbohydrate Res.*, 1973, **31**, 81.
[156] Y. Ghali, A. Youssef, and E. A. El Mobdy, *Phytochemistry*, 1974, **13**, 605.
[157] A. J. Buchala, *Phytochemistry*, 1974, **13**, 2185.
[158] G. N. Richards and R. L. Whistler, *Carbohydrate Res.*, 1973, **31**, 47.
[159] J. Comtat and J. P. Joseleau, *Cellulose Chem. Technol.*, 1974, **7**, 653.
[160] T. Kikuchi and T. Yokotsuka, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2473.
[161] R. Nishino, Y. Ozawa, A. Yasuda, and T. Sakasai, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 291.
[162] J. Comtat, J. P. Joseleau, C. Bosso, and F. Barnoud, *Carbohydrate Res.*, 1974, **38**, 217.
[163] C. M. Stewart, *Cellulose Chem. Technol.*, 1973, **7**, 691.
[164] B. Košiková, J. Polčin, and D. Joniak, *Holzforschung*, 1973, **27**, 59.

geneous fraction of a lignin–carbohydrate complex from acetylated beech wood was studied by ^1H n.m.r. spectroscopy, using methyl 2,3-di-*O*-acetyl-4,6-*O*-veratrylidene-α-D-glucopyranoside and acetylated methanolignin as model compounds.[165] Quantitative analysis of the total number of protons on the aromatic nucleus and of the relative ratio of phenolic and total aliphatic hydroxy-groups showed that three pentose units belong to a trimer of the suggested structural unit of lignin in the isolated fractions of the lignin–polysaccharide complex. Extraction of *Picea excelsa* wood with DMSO released a complex (molecular weight 2.4×10^4) containing 18—21% lignin, which could be separated by gel-permeation chromatography into a number of fractions still containing lignin, as well as residues of D-galactose, D-glucose, D-mannose, L-arabinose, and D-xylose.[166] The polysaccharide of the lignin–carbohydrate complexes isolated from rye grass (*Lolium perenne*) contained mainly D-xylosyl and L-arabinosyl residues.[167] On the basis of the chemical stability of lignin–carbohydrate complexes of *L. perenne*, it was postulated that at least three types of linkage exist between lignin and the carbohydrate moiety; namely, one cleaved on borohydride reduction, another cleaved by alkali, and a linkage resistant to alkali.[168] The carbohydrate portion of the complexes is thought to be composed of β-(1 → 4)-linked D-glucosyl (cellulose) residues and β-(1 → 4)-linked chains of D-xylosyl residues, with chains comprised of L-arabinose and D-galactose linked to O-3 of some of the D-xylosyl residues. Reductive cleavage of the lignin–carbohydrate complex of spruce (*P. excelsa*) wood and its low-molecular-weight form has been achieved using sodium metal in liquid ammonia.[169] Each of the two products was found to be similar in qualitative composition to the protolignin of spruce. The molecular-weight distribution of the decomposition products arising from the carbohydrate portion suggested that lignin creates a linkage between two or more polysaccharide chains in the complex.

Algal Polysaccharides

Alginic Acid.—The block composition of alginates has been characterized by a simple chemical method involving partial hydrolysis with acid, followed by fractional precipitation of the acid-resistant part of the alginate.[170] Alginates from different species of brown algae were examined, and alginates from different tissues were compared in a number of species. The results indicated that young tissue is rich in homopolymeric blocks of D-mannuronic acid, and that differences between alginates from different species are mainly due to alginates from the older parts of the plants. The combined influence of the concentration of sodium carbonate, the time of extraction, and the hydromodulus on the separation and character of alginic acid from *Cystoseira barbata* have been demonstrated.[171] A further illustration of the nearest-neighbour auto-inhibitory effect

[165] B. Košiková, J. Polčin, and D. Joniak, *Cellulose Chem. Technol.*, 1973, **7**, 605.
[166] N. N. Shorygina and N. F. Ehkendieva, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 1393.
[167] I. M. Morrison, *Phytochemistry*, 1974, **13**, 1161.
[168] I. M. Morrison, *Biochem. J.*, 1974, **139**, 197.
[169] N. F. Efendieva, N. N. Shorygina, and N. I. Krasovskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2812.
[170] A. Haug, B. Larsen, and O. Smidsrød, *Carbohydrate Res.*, 1974, **32**, 217.
[171] V. I. Popa and C. I. Simionescu, *Cellulose Chem. Technol.*, 1974, **8**, 153.

in the oxidation of alginate by periodate ions has been presented.[172] It was assumed that both aldehyde groups of the oxidized hexuronic acid residues spontaneously form highly stable, intramolecular hemiacetals with secondary hydroxy-groups on unoxidized residues adjacent to them in the chains. Resolution of some DL-amino-acids by ion-exchange paper chromatography has been achieved by using sodium alginate on silica gel as an adsorbent.[173] Addition of H_4-edta to defined media produced acetylated polyuronides, with a preponderance of D-mannuronic acid residues, in an alginate synthesized by *Azotobacter vinelandii*.[174]

Carrageenan.—A spectrofluorometric procedure has been developed for the assay of carrageenan in the presence of salts, polysaccharides, and proteins; it makes use of the binding of Acridine Orange to the polyanion, which is followed by measurement of the decrease in green fluorescence of the free, monomeric dye in solution.[175] A method for the determination of *i*-carrageenan is based on the colour developed in the reaction of the 3,6-anhydro-D-galactosyl residues with 2-thiobarbituric acid.[176] Methylation analysis of a partially desulphated carrageenan from *Gigartina skottsbergii* confirmed that all the sulphated D-galactosyl residues are linked at O-3 and sulphated at O-4.[177] Observations have suggested that the biologically separate plants of *Chondrus crispus* exhibit distinctive patterns of sulphation of their galactans.[178] The sporophytes were found to add sulphate at O-2 of the precursor, whereas the gametophytes appeared to add it principally at the available O-4 positions, but both types were capable of sulphation at O-6 of the 4-linked D-galactosyl units.

Miscellaneous Algal Polysaccharides.—The ability of chloro-*sym*-triazine dyes to react with glucans, with the formation of covalent bonds, has been used to dye the storage polyglucosides of *Cyanidium caldarium*, *Oscillatoria princeps*, *Rhodymenia pertusa*, and *Spirogyra setiformis* prior to electrophoresis.[179] The technique resolved mixtures of the glucans and defined some of the features of the internal structure of these glucans. Inferences have been made as to the biphyletic evolutionary pathways leading from the Cyanophyceae to the Rhodophyceae and to the Chlorphyceae. A study has shown that the molecular weights, the average number of branch points per molecule, and the ratios of M- and G-chains (mannitol- and glucose-terminating chains) of a number of laminarins of some Far-Eastern brown seaweeds are similar, differing only in details of their fine structure.[180] Insoluble laminarin from *Laminaria hyperborea* has been fractionated by a differential solubility technique into insoluble and soluble fractions, which were then degraded with a purified *exo*-β-(1 $\rightarrow$ 3)-glucanase.[181] Coupled with the results of periodate oxidation, the enzymic studies indicated that the 'insoluble' laminarin is an aggregate of three closely related polysac-

[172] T. Painter and B. Larsen, *Acta Chem. Scand.*, 1973, **27**, 1957.
[173] A. M. Awad and O. M. Awad, *J. Chromatog.*, 1974, **93**, 393.
[174] I. Couperwhite and M. F. McCallum, *Arch. Mikrobiol.*, 1974, **97**, 73.
[175] R. B. Cundall, G. O. Phillips, and D. P. Rowlands, *Analyst*, 1973, **98**, 857.
[176] W. Anderson and W. Bowtle, *Analyst*, 1974, **99**, 178.
[177] A. S. Cerezo, *Carbohydrate Res.*, 1974, **36**, 201.
[178] E. L. McCandless, J. S. Craigie, and J. A. Walter, *Planta*, 1973, **112**, 201.
[179] J. F. Fredrick, *Plant and Cell Physiol.*, 1973, **14**, 1187.
[180] L. A. Elyakova and T. N. Zvyagintseva, *Carbohydrate Res.*, 1974, **34**, 241.
[181] T. E. Nelson and B. A. Lewis, *Carbohydrate Res.*, 1974, **33**, 63.

charides, *viz.* a soluble, branched, reducing component, an insoluble, linear, reducing component, and a linear, non-reducing component having a mono-substituted D-mannitol residue as the end group. Laminarin from *Eisenia bicyclis*, which appears to be a linear glucan containing β-1,3- and β-1,6-linkages, has been hydrolysed with a β-1,6-D-glucan 6-glucanohydrolase, with the release of a series of 6-*O*-β-laminarioligosylglucoses, laminaribiose, and gentiobiose.[182] Aqueous solutions of laminarin have been subjected to γ-irradiation.[183] On irradiation in the presence of oxygen, the polysaccharide underwent oxidative degradation, whereas mono- and oligo-saccharides were released in the presence of nitrous oxide. On the basis of i.r. and enzymic evidence, the water-soluble cytoplasmic polysaccharides from *Achyla ambisexualis* and *A. heterosexualis* have been characterized as β-(1 → 3)-D-glucans.[184] During the vegetative phase of the life-cycle, the glucan accumulates in the cytoplasm. A starch-type polysaccharide, which gave D-glucose as the only product from the action of glucoamylase, has been found in *Platymonas* sp.[185] Reticuloendothelial-stimulating activity has been found in extracts of a commercial *Chlorella* preparation.[186] The factor was purified and identified as a polysaccharide (molecular weight 1.3×10^3) that could be degraded by a fungal β-1,3-glucanase, releasing D-glucose as the only product. Laminarin containing 1.7% D-mannitol end-groups and fucans with a relatively high proportion of D-galactose have been isolated from the brown seaweed *Desmarestia aculeata*.[187] A number of oligosaccharides (13)—(19) have been isolated and characterized from hydrolysates of the sulphated polysaccharide obtained from *Aeodes ulvoidea*.[188]

β-D-Gal*p*-(1 → 6)-D-Gal
(13)

β-D-Gal*p*-(1 → 4)-D-Gal
(14)

4-*O*-Me-α-L-Gal*p*-(1 → 6)-D-Gal
(15)

2-*O*-Me-α-D-Gal*p*-(1 → 3)-D-Gal
(16)

β-D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal
(17)

β-D-Gal*p*-(1 → 4)-[4-*O*-Me-α-L-Gal*p*-(1 → 6)]-D-Gal
(18)

6-*O*-Me-β-D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal
(19)

[182] Y. Shibata, *J. Biochem. Japan*, 1974, **75**, 85.
[183] L. I. Kudryashov, S. M. Yarovaya, T. N. Zvyagintseva, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1974, **44**, 1393.
[184] S. Faro, *Amer. J. Bot.*, 1972, **59**, 919.
[185] H. Suzuki, *Phytochemistry*, 1974, **13**, 1159.
[186] M. Kojima, M. Dobashi, and S. Ino, *J. Reticuloendothelial Soc.*, 1973, **14**, 192.
[187] E. Percival and M. Young, *Carbohydrate Res.*, 1974, **32**, 195.
[188] A. J. R. Allsobrook, J. R. Nunn, and H. Parolis, *Carbohydrate Res.*, 1974, **36**, 139.

A series of acidic polysaccharides (molecular weights 5×10^3—2×10^5) containing *O*-sulphate groups and displaying calcium-binding properties has been isolated from the calcareous alga *Halimeda opuntia*.[189] Desulphation and methylation have shown that L-fucosyl, D-xylosyl, and D-galactosyl residues occupy non-reducing, terminal positions and that D-galactose and D-mannose occupy branch points in the brown-seaweed polysaccharides pelvetian and sargassan. From analysis of fragments obtained by Smith degradation of pelvetian and of its degradation product, it was concluded that the parent polysaccharide is composed of a periodate-resistant core of D-mannose, D-glucuronic acid, and, probably, D-xylose, with peripheral L-fucosyl residues that are generally sulphated.[190, 191] The acid-extractable, water-soluble polysaccharides from *Sargassum linifolium* have been fractionated to give a neutral, laminarin-type glucan and a sulphated heteropolysaccharide (sargassan), which is composed of D-glucuronic acid, D-mannose, D-galactose, D-xylose, L-fucose, and protein.[192] Sulphate groups have been detected on some of the D-galactosyl and L-fucosyl residues.[193] Evidence for the existence of a (1 → 4)-linked β-D-glucuronic acid and β-D-mannose backbone in sargassan was obtained from a study of the behaviour of the polysaccharide towards periodate before and after partial acid hydrolysis, alkali treatment, and methanolysis.[194] The heteropolymer also contains partially sulphated branches attached to the backbone, and these branches comprise various proportions of (1 → 4)-linked β-D-galactosyl, β-D-galactosyl 6-sulphate, and β-D-galactosyl 3,6-disulphate residues, (1 → 2)-linked α-L-fucosyl 4-sulphate residues, and (1 → 3)-linked β-D-xylosyl residues. The cell wall of *Scenedesmus obliquus* is comprised of residues of D-glucose, D-galactose, D-mannose, L-arabinose, L-fucose, and L-rhamnose.[195] Autoradiography using [^{34}S]-sulphate has revealed that the Golgi complex is the site of sulphation of polysaccharides in the brown seaweed *Laminaria*.[196] Funoran, a polysaccharide isolated from *Gloiopeltis tenax*, has been fractionated into three components by precipitation of the cetyl pyridinium chloride complex with potassium chloride; each of the components exhibited a different ratio of D-galactose, 3,6-anhydro-D-galactose, and ester sulphate.[197] Agaropectin can be removed from samples of agar by treatment with cetyl pyridinium chloride.[198] The biosynthesis of algal polysaccharides has been reviewed.[199]

[189] E. L. Böhm, *Internat. Rev. Ges. Hydrobiol.*, 1973, **58**, 117.
[190] A. F. Pavlenko, A. V. Kurika, V. A. Khomenko, and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1974, 172.
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4
Microbial Polysaccharides

BY R. J. STURGEON

Bacterial Cell Walls and Membranes

The chemical structures and immunological responses of Gram-positive and Gram-negative organisms have been reviewed with respect to antigenic activity and the factors affecting wall lysis.[1] The basic compositions of lipopolysaccharides, polysaccharides, and mucopeptides were also reviewed. A review on the structure and the function of the cell envelope of Gram-negative bacteria has dealt with the peptidoglycan–lipoprotein complexes, the synthesis of the cell wall, the association of enzymes with specific cell-wall components, and the functions of enzymes associated with the cell wall.[2] Recent advances concerning the interaction of penicillin with bacterial cell walls, with respect to penicillin-binding and penicillin-sensitive enzymes, have been reported.[3]

Reports from a symposium on the action of antibiotics on microbial walls and membranes have been published.[4] The biochemistry of bacterial cell-wall envelopes has been covered in a review dealing with the structures, the biosynthesis, and the biological significance of teichoic acids, with reference to their localization in the wall and their association with other wall polymers.[5] Another review has considered the effects of antibiotics on the biosynthesis of teichoic acids and the antigenic properties of the polymers.[6]

Teichoic Acids.—A report has detailed a number of the conditions necessary for the occurrence of concanavalin A–teichoic acid interactions.[7] The teichoic acids of two strains of *Actinomyces rimosus* are localized in the Actinomycete cell wall, and were shown to have similar structures by comparison of the products of acidic and alkaline hydrolyses.[8] Particulate enzyme preparations obtained from *Bacillus stearothermophilus* were found to catalyse the synthesis of teichoic acids.[9] With CDP-glycerol as the sole substrate, the preparations synthesized 1,3-poly(glycerol phosphate), and pulse-labelling experiments showed that the enzyme system transferred glycerol phosphate units to the glycerol end of the chain in an irreversible reaction. When the enzyme system from *B. stearothermo-*

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philus was supplied with both CDP-glycerol and UDP-D-glucose, 1-D-glucosyl-2,3-poly(glycerol phosphate) was synthesized in addition to the 1,3-isomer. It was suggested that the biosynthetic pathways of the two poly(glycerol phosphates) may share a rate-limiting step, since the proportion of the 2,3-isomer in the products increased with increasing amounts of UDP-D-glucose although the total glycerol phosphate incorporated into the products remained constant. Two very poor lytic mutants of *B. licheniformis*, which had neither teichuronic acid nor D-glucose in their walls, when grown with an insufficient supply of inorganic phosphate ceased to make teichoic acid; the walls contained greatly increased levels of mucopeptide.[10] Phosphoglucomutase has been isolated from *B. subtilis* in an effort to determine whether this enzyme could be involved in regulation of the biosynthetic pathway leading to the D-glucosylation of the teichoic acid.[11] Two dimeric forms of phosphoglucomutase were found to exist, each with an activity that was markedly inhibited by GTP and UTP, but not by other nucleotides.[12] Teichoic acids from *B. subtilis* exhibited a higher sedimentation rate after affinity chromatography on columns of concanavalin A–Sepharose than preparations obtained by conventional methods.[13] In dilute buffers and in water, the teichoic acid of *B. subtilis* was shown to possess a rigid-rod or extended conformation, but this secondary structure was lost in the presence of high concentrations of salts, which led to the adoption of a random-coil conformation.[14] Electron microscopy has shown that the cell walls of *B. licheniformis* and *Staphylococcus aureus* possess a trilammelar density distribution.[15] Removal of the teichoic and teichuronic acids indicated that the density distribution is not due to discrete layers of the various components, but rather to variations in the packing material throughout the wall. Extracts of whole bacteria of *B. pumilus*, *Lactobacillus plantarum*, *Staph. aureus*, and *Streptomyces faecium* were found to precipitate with *Haemophilus influenzae* antiserum, because the poly(ribitol phosphate) component of the bacteria reacted with antibodies to the capsular polysaccharide present in the antiserum.[16] Preliminary analysis of the capsular polysaccharide revealed the presence of ribitol and ribose, thus providing an explanation for the cross-reaction. The synthesis of the teichoic acids has been shown to be severely affected in a methicillin-requiring strain of *Pediococcus cerevisiae*.[17] In agreement with previous serological findings with whole bacteria, three types of cell-wall polysaccharides from animal, coagulase-positive Staphylococci have been recognized according to their serological and chemical characteristics.[18] All three types appeared to be glycerol teichoic acids having 2-acetamido-2-deoxy-D-glucose and D-glucose, D-mannose and D-glucose, and D-galactose as the respective antigenic determinants. The wall teichoic acids of a large number of *Staph. aureus* strains from animals have been shown to be hetero-

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geneous, and they could be classified into three groups.[19] The linkage between the lipoteichoic acid carrier from *Staph. aureus* and the poly(ribitol phosphate) synthesized with poly(ribitol phosphate)polymerase has been shown to be a phosphoric diester bond between a glycerol residue in the carrier molecular and a ribitol residue.[20] Lipoteichoic acid of *Staph. aureus* mesosomal vesicles has been characterized chemically.[21] A working model has been proposed in which DNA attached to the membrane sequesters Mg^{2+} ions, with activation of the adjacent plasma membrane to synthesize lipoteichoic acid, which in turn sequesters more Mg^{2+} ions. The presence of an increased, localized concentration of Mg^{2+} ions activates the enzymes in an adjacent membrane to synthesize the new membrane with elevated levels of phospholipid and lipoteichoic acid. This forms a mesosomal vesicle, which, because of the active synthesis of plasma membrane and cell wall, produces a cross-wall septum. The techoic acid extracted with phenol from group A Streptococci has been found to possess DL-glycerol 1-phosphate, and not 2-acetamido-2-deoxy-D-glucose, as the major antigenic determinant.[22] The cross-reactivity of the membrane teichoic acid of *Streptococcus faecalis* with anti-pneumococcal type XII sera is due to the presence of multiple kojiobiosyl residues in the teichoic acid, and the cross-reaction confirmed the presence of these residues in the capsular polysaccharide S XII.[23] The ultrastructural localization of teichoic acids of *S. faecalis* has been achieved by means of two direct methods and one indirect method, each involving interaction with concanavalin A.[24] All three methods indicated a uniform distribution of the teichoic acids. Two serologically active antigens have been isolated from *S. mutans* and separated from each other.[25] Alkaline hydrolysis of one of the antigens afforded products typical of those released from glycerol teichoic acids. Side-chains were found to be composed of mono- and tri-galactosyl moieties. The membrane and cell-wall lipoteichoic acids of *S. sanguis* were found to have similar structures, consisting of glycerol moieties linked by 1,3-phosphoric diester bonds.[26] Six heteroglycans containing glycerol phosphate from *S. sanguis* were found to be composed of L-rhamnose, D-glucose, and 2-acetamido-2-deoxy-D-glucose in similar proportions, but the contents of glycerol and phosphorus were different.[27] The polysaccharides probably originate from the same cell-wall polysaccharide, being produced as a result of cleavage of the phosphoric diester bonds during extraction. Preliminary studies showed that the glycerol moiety is probably linked to the polysaccharide through a phosphoric diester bond.[28]

Peptidoglycans.—A method for the quantitative determination of muramic acid is based on the liberation of lactic acid and its conversion into acetaldehyde,

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which can be determined with *p*-hydroxybiphenyl reagents.[29] Chemical analyses of the cell walls of organisms isolated from cases of lepromatous and tuberculoid leprosy have made possible their assignment to the genera *Corynebacterium*, *Mycobacterium*, or *Propionibacterium*.[30] The cell walls were shown to contain 2-amino-2-deoxy-D-glucose, muramic acid, and neutral hexoses. *X*-Ray diffraction, i.r., and density measurements of the mureins of the Gram-negative organism *Spirillum sepens* and the Gram-positive organism *Lactobacillus plantarum* have provided data which, together with theoretical considerations and model building, have led to the proposal that the structure of the glycan chains is similar to those of chitin and cellulose.[31] The peptides are composed of alternating D- and L-amino-acids having the 2.2-helical conformation, with a linkage to the carbohydrate chains through the N-terminus and forming two hydrogen bonds with the sugar residues. This linkage produces an angle of about 150° between the carbohydrate and peptide chains. The action of glycine in inhibiting growth was studied with eight different species of various genera.[32] Glycine was incorporated into the nucleotide-activated, peptidoglycan precursors, with the level of incorporation equivalent to the decrease in the amount of alanine; in the majority of cases, glycine was also incorporated into the peptidoglycan, replacing L-alanine in position 1 and D-alanine in positions 4 and 5 of the peptide sub-unit. Two mechanisms leading to a more-loosely cross-linked peptidoglycan and to morphological changes of the cells were considered; one in which the accumulation of glycine-containing precursors may lead to the normal balance between polysaccharide synthesis and controlled enzymic hydrolysis during growth, and the other in which the modified glycine-containing precursors may be incorporated. Since these are poor substrates in the transpeptidation reaction, a high percentage of mucopeptides would not be cross-linked.

UDP-2-acetamido-2-deoxy-D-glucose pyrophosphorylase from *Bacillus licheniformis* was inhibited by UDP-Mur*N*Ac-pentapeptide (UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-diaminopimelyl-D-alanyl-D-alanine) and by CDP-glycerol, whereas CDP-glycerol pyrophosphorylase was inhibited by UDP-Mur*N*Ac-pentapeptide and was stimulated by UDP-2-acetamido-2-deoxy-D-glucose.[33] The possible role of this type of interaction, which involves a precursor of one cell-wall polymer and an enzyme involved in the synthesis of the precursor of a second polymer, was discussed. Cell-free membrane preparations from a poor lytic mutant of *B. licheniformis* produced a non-cross-linked, radioactive peptidoglycan on incubation with UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose or *meso*-diamino-[^{14}C]pimelic acid.[34] From chemical degradation, it was concluded that the glycan chain grows by extension at the reducing end while remaining attached by an acid-labile linkage (*e.g.* a glycosyl linkage) to undecaprenol pyrophosphate. The synthesis of peptidoglycan by cell-free membrane and cell-wall membrane preparations from an autolysin-deficient, β-lactamase-negative mutant of *B.*

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licheniformis was studied.[35] The membrane preparation synthesized a polymer that was not cross-linked and whose formation was not inhibited by β-lactam antibiotics. Although bacitracin inhibited peptidoglycan synthesis, synthesis was not restricted to the addition of one disaccharide–pentapeptide unit at each synthetic site, an average of 2—3 such units being added. D-Alanine carboxypeptidase was not inhibited by bacitracin. Peptidoglycan synthesis was three- or four-fold more sensitive to vancomycin than was the release of D-alanine by the action of carboxypeptidase. A cell-wall membrane preparation from *B. licheniformis* was able to incorporate radioactivity from a peptidoglycan precursor having an amino-group of diaminopimelic acid blocked by a [^{14}C]acetyl group.[36] The enzymically degraded product contained cross-linked dimers, indicating that newly synthesized peptidoglycan chains had been cross-linked to the pre-existing cell wall. The synthesis of the cell wall of *B. megaterium* and the degradation of the mucopeptide by lysozyme under different physiological conditions have been studied.[37] The resistance to lysozyme diminished after transfer of the stationary cells to a fresh medium, and was also facilitated by the turnover of cell wall. The immunostimulatory properties of lysozyme-hydrolysed *B. megaterium* have been investigated.[38] The results suggested that the reticulated structure of the peptidoglycan is a factor that contributes to the immunostimulatory activity of the walls. The extent and rate of cross-linking of diaminopimelic acid to peptidoglycan have been determined by the reaction of nitrous acid with unprotected amino-groups of non-cross-linked diaminopimelic acid.[39] This method has also been used to demonstrate the sensitivity of the cross-linking reaction to penicillin *in vivo*. Cells of *B. megaterium* were able to use such externally added precursors as UDP-2-acetamido-2-deoxy-D-glucose and UDP-Mur*N*Ac-pentapeptide to synthesize peptidoglycan, if the bacteria were first treated with toluene.[40] The precursors could be polymerized to form peptidoglycan, which was attached to the pre-existing cell wall by peptide cross-bridges. Peptidoglycan synthesized *in vitro* by membrane preparations of *B. megaterium* had a molecular weight in excess of 6×10^6 and exhibited a buoyant density in caesium chloride solution different from that of the native cell wall.[41] This newly made material was not covalently attached to substantial amounts of previously formed cell material, such as cell wall. In the presence of penicillin, a peptidoglycan of much lower molecular weight was synthesized, suggesting that transpeptidation was responsible, in part, for the large size of the product. In synchronously sporulating cells of *B. sphaericus*, the specific activities of the enzymes required for the synthesis of UDP-Mur*N*Ac-pentapeptide precursors of the vegetative cell-wall peptidoglycan decayed by 50% after the end of exponential cell division, whereas the activities of L-alanine and D-alanyl-D-alanine ligases and D-alanyl-D-alanine

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synthetase increased in parallel with the appearance of diaminopimelyl ligase.[42] Preparations of *B. stearothermophilus* catalysed extensive cross-linking of enzymically synthesized peptidoglycan after incubation with UDP precursors.[43] In the presence of ATP and either ammonia or L-glutamine, amidation of the peptidoglycan also occurred. A number of antibodies have been shown to inhibit the synthesis of peptidoglycan by particulate enzyme preparations from *B. stearothermophilus* and *E. coli*, with the accumulation of lipid intermediates.[44] Chemical analyses of the material excreted by a biotin-requiring mutant of *Brevibacterium divaricatum* in the presence of penicillin showed that the main constituents were peptidoglycan components of a non-cross-linked structure bearing both L- and D-alanine residues; evidence was also obtained for the occurrence of extractable lipid material, non-amino-sugars, and organic phosphate.[45] As a result of labelling experiments with L-[^{14}C]glutamic acid and the analysis of lysozyme digests, it was suggested that some of the components might contain mucopeptide fragments covalently linked to a polysaccharide. Electron microscopy of the solubilized peptidoglycans from *Brucella abortus* and *B. melitensis* showed them to consist of three layers.[46] The walls of *Corynebacterium poinsettiae* and *C. betae* contain, in addition to phosphate-free polysaccharides, a peptidoglycan that contains phosphate, some of which is present as muramic acid phosphate.[47] Similar morphological changes of the cell envelope of *E. coli* were observed during conditions of 2-amino-2-deoxy-D-glucose starvation and penicillin treatment.[48] The results seem to indicate that amino-sugar starvation disrupted the synthesis of the peptidoglycan. Penicillin-resistant, temperature-sensitive mutants of *E. coli* that synthesize hypo- or hyper-cross-linked peptidoglycans have been isolated.[49] Studies of revertants indicated that penicillin resistance, temperature sensitivity, cross-linking, growth characteristics, and morphological changes may be related to a single mutational event in both of these groups. A purified hydrolase from phage λ has been used to degrade *E. coli* and *Salmonella typhi* cell walls to dialysable products.[50] One of the fragments has been characterized as 2-acetamido-2-deoxy-β-D-glucose-(1 $\rightarrow$ 4)-1,6-anhydro-*N*-acetylmuramic acid. Enzymes involved in the cross-linking of the wall peptides of *E. coli* K12 have been investigated.[51] There apparently exist in the membranes a DD-carboxypeptidase–endopeptidase system, which catalyses the hydrolysis of UDP-*N*-acetylmuramyl-pentapeptide to the corresponding tetrapeptide, and a DD-carboxypeptidase–transpeptidase system. The sensitivity to β-lactam antibiotics of the enzymes involved in cross-linking of the wall peptides in *E. coli* has been reported.[52] A membrane system

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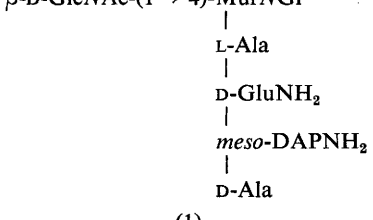
having a high specificity for peptidoglycan synthesis in *Gaffkya homari* has been described.[53] The specificity profile towards the peptide subunit of UDP-*N*-acetylmuramyl-pentapeptide was defined for the overall reaction and compared with that of the phospho-*N*-acetylmuramyl-pentapeptide translocase. Although this enzyme is considered to be a key one in the selection of precursors, an additional specificity barrier in the enzyme system that catalysed the biosynthesis of peptidoglycan was suggested. A constituent of the cell wall of *Halococcus* sp. has been identified as 2-amino-2-deoxyguluronic acid.[54] Intact cells of *Micrococcus luteus* incorporated radioactive L-lysine into the cell-wall peptidoglycan, but the incorporation was inhibited by penicillin G, with no formation of cross-linkages *via* ε-amino-groups of the lysine residues.[55] Digestion of the cell walls of *M. lysodeikticus* with *endo-N*-acetylglucosaminidase and *endo-N*-acetylmuramidase liberated tetra- and hexa-saccharides composed of *N*-acetylmuramyl-2-acetamido-2-deoxy-D-glucose repeating units and hexa- and octasaccharides composed of 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramic acid repeating units.[56] The disaccharide-tetrapeptide (1), isolated from *Mycobacterium smegmatis*, has an adjuvant activity towards circulating antibodies to ovalbumin, and it induces a delayed hypersensitivity toward this antigen.[57]



(1)

The minimum structural requirement for adjuvant activity for mycobacterial peptidoglycan was shown to be *N*-acetylmuramyltripeptide.[58] A water-soluble peptidoglycan having adjuvant activity has been isolated from *M. smegmatis* and *M. tuberculosis* after removal of a neutral arabinogalactan.[59] Cross-linking between peptide units in the wall peptidoglycan of *M. smegmatis* was shown to be mediated through D-alanyl-(D)-*meso*-diaminopimelic acid and *meso*-diaminopimelyl-*meso*-diaminopimelic acid linkages, occurring in the ratio of 2 : 1.[60] Examination of the primary structures of the peptidoglycans of eight strains of Peptococcus indicated that four types of peptidoglycan exist, with differences

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occurring in the nature and positions of amino-acids in the peptide subunits.[61] The purification and characterization of serologically active cell-wall components from a number of Planococcus strains have been reported.[62] Quantitative agglutination studies with anti-peptidoglycan antiserum revealed that, although Planococcus peptidoglycans share antigenic determinants with Staphylococcus peptidoglycans, they also have determinants of their own. The peptidoglycans isolated from *Proteus vulgaris*, *Moraxella glucidolytica*, and *Neisseria perflava* possess adjuvant activity for the late hypersensitivity reaction, but, although all three polymers appear to have the same chemical structure, their effect was different from that observed with Mycobacteria.[63] The sequence H_2N-L-Ala-D-Glu-A_2pm-D-Ala-CO_2H (A_2pm = 2,5-diaminopimelate) has been reported for the structure of the peptide side-chain of the peptidoglycan of *Pseudomonas aeruginosa*.[64] The peptidoglycan of *P. solanacearum* contains *N*-acetylmuramic acid, 2-acetamido-2-deoxy-D-glucose, alanine, and glutamic acid, with the amino-acids in the ratio of 1 : 1 rather than 1 : 2, which is more usual.[65] Cell-free preparations of the vegetative cells of *Sporosarcina ureae* required UDP-*N*-acetylmuramoylpentapeptide and UDP-2-acetamido-2-deoxy-D-glucose for synthesis.[66] In the presence of glycyl-t-RNA, D-glutamate, and ATP, both amino-acids were incorporated into the γ-glutamylglycyl interpeptide bridge; the peptidoglycan was shown to be cross-linked. In a study of antigenic specificity, at least three determinants have been located in the peptide subunits of the mucopeptide of *Staphylococcus aureus*.[67] A different mode of cleavage, which is dependent on ADP, has been suggested for the lysine-containing UDP-*N*-acetylmuramylpentapeptide.[68] Extracts from *Staph. aureus* and *Staph. faecalis* released D-Ala and D-Ala-D-Ala from the peptide, possibly as a consequence of a reversal of the enzyme UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-L-lysyl:D-alanyl-D-alanine ligase (ADP-forming). A functional regulatory mechanism was suggested in which the level of lysine-containing UDP-muramylpentapeptide depended on the intracellular ATP/ADP ratio. The inhibition of Staphylococcus phages by mucopeptide preparations has been reported.[69] The specificity profile of phospho-*N*-acetylmuramylpentapeptide translocase (UDP-Mur*N*Ac-Ala-γ-D-Glu-Lys-D-Ala-D-Ala:undecaprenylphosphate phospho-*N*-acetylmuramylpentapeptide transferase) has been shown to be consistent with the effects *in vivo* of elevated concentrations of glycine on the growth of *Staph. aureus*.[70] It was suggested that the translocase has a key role in selecting analogues of UDP-Mur*N*Ac-Ala-γ-D-Glu-Lys-D-Ala-D-Ala for use in peptidoglycan synthesis. Preparations from lysozyme and muramidase digests of groups A and B streptococcal cell walls contained a range of components of molecular sizes from

[61] K. H. Schleifer and E. Nimmermann, *Arch. Mikrobiol.*, 1973, **93**, 245.
[62] C. Endresen and P. Oeding, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 571.
[63] C. Nauciel, J. Fleck, J. P. Martin, and M. Mock, *Compt. rend.*, 1973, **276**, *D*, 3499.
[64] H. D. Heilmann, *European J. Biochem.*, 1974, **43**, 35.
[65] T. J. Wacek and L. Sequeira, *Physiol. Plant Pathol.*, 1973, **3**, 363.
[66] P. E. Linnett, R. J. Roberts, and J. L. Strominger, *J. Biol. Chem.*, 1974, **249**, 2497.
[67] S. Helgeland, A. Grov, and K. H. Schleifer, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 413.
[68] B. Oppenheim and A. Patchornik, *F.E.B.S. Letters*, 1974, **48**, 172.
[69] U. Beige, G. Kemmer, and G. Seltmann, *Z. allgem. Mikrobiol.*, 1973, **13**, 553.
[70] W. P. Hammes and F. C. Neuhaus, *J. Biol. Chem.*, 1974, **249**, 3140.

20×10^6 to less than 5×10^3.[71] Larger fragments reacted serologically as precipitinogens, and smaller fragments as haptenic inhibitors, with rat peritoneal cells. Cell walls prepared from parental and filamentous cells of *Streptococcus cremoris* contained 2-amino-2-deoxy-D-glucose, muramic acid, 2-amino-2-deoxy-D-glucose 6-phosphate, D-glucose, D-galactose, and L-rhamnose, as well as aspartic acid, glutamic acid, alanine, and lysine.[72] Enzymic and chemical analyses revealed that the glycan strands are incompletely substituted and peptide cross-bridging is not mediated by D-alanyl-D-alanyl linkages. *N*-Substitution of the free amino-groups of the amino-sugars by dinitrophenylation or by acetylation enhanced the response of the peptidoglycan to digestion by lysozyme. A pentapeptide containing a *C*-terminal D-alanyl-D-alanine moiety (Gly-L-Ala-L-Ala-D-Ala-D-Ala) has been synthesized and rendered immunogenic by coupling to a protein carrier.[73] The cross-reactivity of antibodies towards the conjugate with solubilized peptidoglycan and the reaction between the conjugate and Streptococcus group A variant antiserum have been reported. Although the cell wall of *Sulpholobus acidocaldarius* contains lipoprotein and small amounts of carbohydrate and amino-sugars, the cell wall of the subunit is devoid of peptidoglycan.[74]

Lipopolysaccharides.—Lipopolysaccharide preparations with very different sugar moieties gave similar results in stimulating density-inhibited, chick-embryo fibroblasts, indicating that the architecture of the hydrophilic carbohydrate part of the molecule is not critical for the mitogenic effect.[75] Lipoglycoprotein was shown to prevent the attachment to erythrocytes of unheated and heated lipopolysaccharides of smooth and rough strains of all Gram-negative bacteria tested.[76] The properties and activity of the lipopolysaccharide receptor from human erythrocytes have been reported.[77, 78]

The micellar structures and endotoxic activities of Gram-negative lipopolysaccharides have been investigated.[79] The chemical nature of the lipopolysaccharide of *Escherichia coli* 014:K7 has been examined.[80] As a result of genetic and immunochemical studies, it was demonstrated that this strain is an encapsulated R-mutant with a complete lipopolysaccharide core, but lacking O-specific polysaccharides. Peritoneal exudate macrophages produced collagenase when exposed to lipopolysaccharides isolated from *E. coli* and *Salmonella minnesota* in culture.[81] Lipid-containing fractions of lipopolysaccharides, including a glycolipid

[71] B. Heymer, B. Bultmann, W. Schachenmayer, R. Spanel, O. Haferkamp, and W. C. Schmidt, *J. Immunol.*, 1973, **111**, 1743.
[72] K. G. Johnson and I. J. McDonald, *Canad. J. Microbiol.*, 1974, **20**, 905.
[73] K. H. Schleifer and P. H. Seidl, *European J. Biochem.*, 1974, **43**, 509.
[74] R. L. Weiss, *J. Bacteriol.*, 1974, **118**, 275.
[75] A. Vaheri, E. Ruoslanti, M. Sarvas, and M. Nurminen, *J. Exp. Med.*, 1973, **138**, 1356.
[76] G. F. Springer, J. C. Adye, A. Bezkorovainy, and B. Jirgensons, *Biochemistry*, 1974, **13**, 1379.
[77] G. F. Springer, J. C. Adye, A. Bezkorovainy, and J. R. Murthy, *J. Infectious Diseases*, 1973, **128**, 5202.
[78] G. F. Springer, J. C. Adye, A. Bezkorovainy, and B. Jirgensons, *Biochemistry*, 1974, **13**, 3202.
[79] M. Niwa, T. Hiramatsu, Y. Kuwajuma, and H. Shimouchi, *Japan J. Med. Sci. Biol.*, 1973, **26**, 20.
[80] G. Schmidt, B. Jann, and K. Jann, *European J. Biochem.*, 1974, **42**, 303.
[81] L. M. Wahl, S. M. Wahl, S. E. Mergenhagen, and G. R. Martin, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 3598.

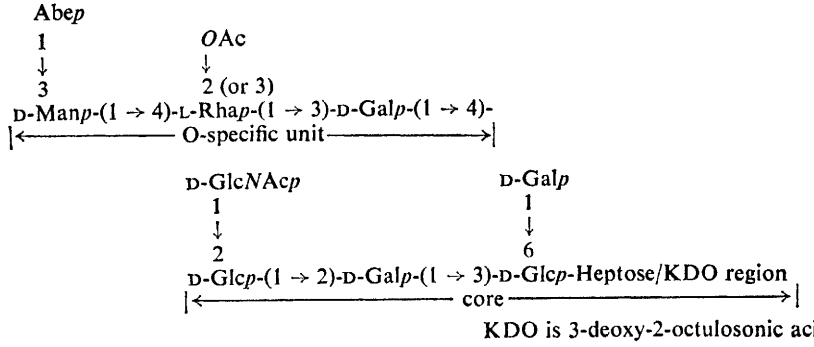
from a rough mutant of *S. minnesota*, and lipid A were potent stimulators, but lipid-free polysaccharides produced no effect. The purified precursor of the Hageman factor (factor XII) involved in blood clotting was found to bind the soluble lipopolysaccharide of *E. coli*.[82] The activation capacity was found to reside in the lipid-A region of the molecule. In structural studies on *Klebsiella* O group 7 lipopolysaccharide, a modification of the Smith degradation was used in which the polyalcohol, obtained after periodate oxidation and reduction, was methylated before, and ethylated after, mild hydrolysis with acid.[83] The product obtained was then hydrolysed and the components were analysed as the alditol acetates by g.l.c.–m.s. It was concluded that the side-chains are composed of the tetrasaccharide repeating unit (2), although there is still some uncertainty over the anomeric

$$\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Rib}f\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow \quad (2)$$

configuration of one of the four sugar residues in the structure. The vegetative cells of *Myxococcus fulvus* produce a lipopolysaccharide composed mainly of D-mannose, D-galactose, D-glucose, L-rhamnose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose, with minor amounts of L-ribose, D-xylose, L-arabinose, 3-*O*-methyl-D-xylose, and 3-deoxy-2-octulosonic acid (KDO).[84] The enzymic synthesis of the CDP-3,6-dideoxyhexoses occurring in Salmonellae and in five serotypes of *Pasteurella pseudotuberculosis* has been shown to involve CDP-6-deoxy-D-*xylo*-hexos-4-ulose, pyridoxamine 5′-phosphate, and NADP(H).[85] Further reduction of CDP-6-deoxy-D-*xylo*-hexos-4-ulose with NADP(H) and another enzyme furnished CDP-3,6-dideoxy-D-*erythro*-hexos-4-ulose.[86] A reversible dissociation of the protein–lipopolysaccharide complex of *Pseudomonas aeruginosa*, after treatment with sodium deoxycholate, was demonstrated by ultracentrifugation, gel filtration, and immunological techniques.[87] A correlation has been demonstrated between the presence of 2-amino-2,6-dideoxy-D-glucose, which is located in the side-chain of the lipopolysaccharide of *P. aeruginosa*, and the sensitivity towards the bacteriocin piocyin.[88] The phosphorylated polysaccharide isolated from the lipopolysaccharide of *P. alcaligenes* has been shown to contain D-glucose, L-rhamnose, L-*glycero*-D-*manno*-heptose, 2-amino-2-deoxy-D-galactose, alanine, 3-deoxy-2-octulosonic acid, and phosphorus.[89] By the application of classical structural methods, it was deduced that all the L-rhamnosyl residues and part of the D-glucosyl and heptosyl residues occur as non-reducing termini. Other residues are substituted at O-3, and most heptosyl residues are esterified with phosphate, possibly at O-4. Serological investigations carried out with isolated O-antigens from twelve strains of *Rhodopseudomonas palustris* have shown that the antigens could be arranged into three distinct serotypes, which

[82] D. C. Morrison and C. G. Cochrane, *J. Exp. Med.*, 1974, **140**, 797.
[83] B. Lindberg, J. Lönngren, W. Nimmich, and U. Rudén, *Acta Chem. Scand.*, 1973, **27**, 3787.
[84] G. Rosenfelder, O. Lüderitz, and O. Westphal, *European J. Biochem.*, 1974, **44**, 411.
[85] P. A. Rubenstein and J. L. Strominger, *J. Biol. Chem.*, 1974, **249**, 3776.
[86] P. A. Rubenstein and J. L. Strominger, *J. Biol. Chem.*, 1974, **249**, 3782.
[87] N. Rubio, A. Portolés, and R. Lopez, *Arch. Mikrobiol.*, 1973, **94**, 149.
[88] N. Suzuki, *F.E.B.S. Letters*, 1974, **48**, 301.
[89] J. A. Lomax, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1974, **139**, 633.

are identical with three chemotypes previously established.[90] An amino-sugar, characterized as 2-amino-2-deoxy-6-*O*-methyl-D-glucose, has been shown to be part of the O-specific side-chain, and not the lipid-A portion, of the lipopolysaccharide of *R. palustris* strains.[91] All *R. viridis* lipopolysaccharides investigated have been shown to belong to the same chemotype, having polysaccharide moieties composed of 3-*O*-methyl-L-xylose, 3-*O*-methyl-D-mannose, D-mannose, D-galactose, and D-glucose, in addition to 3-deoxy-2-octulosonic acid, 2-amino-2-deoxy-D-glucose, 2-amino-2,6-dideoxy-D-glucose (quinovosamine), and 2-amino-2-deoxy-D-galacturonic acid.[92] The *R. viridis* O-antigens are clearly distinguishable from the corresponding antigens of *R. palustris* by the lack of heptosyl residues. Like lipid A from *R. palustris*, but unlike lipid A of Enterobacteriaceae, that derived from *R. viridis* contains a 2,3-diamino-2,3-dideoxyhexose in place of 2-amino-2-deoxy-D-glucose. The responsibility for agglutination in bacteria is frequently attributed to either polysaccharides or lipopolysaccharides, but other results have indicated that a more important role in agglutination must be attributed to proteins; it was suggested that, since proteins rather than polysaccharides are common to Salmonella and *E. coli*, the particular antigens involved in agglutination tests should be ascertained bearing in mind that the bacteria contain many proteins on the surface.[93] A hybrid of two Salmonella groups, B and C_2, has been produced, and its cell-wall lipopolysaccharide was analysed.[94] The hybrid possessed the *rfb* gene cluster from group B, which determined the synthesis of O-specific units with the structure abequosyl-D-mannosyl-L-rhamnosyl-D-galactose. Other genes affecting lipopolysaccharide synthesis were of group C_2 origin and, thus, the lipopolysaccharide consisted of a C_2-determined core with single, B-determined O-specific units. The B-type O-specific unit of the hybrid was modified by an *O*-acetyl group attached to an L-rhamnosyl residue, a modification seen in the group-C_2 parent but not in the group-B parent. From methylation analysis and determination of the positions of alkali-labile groups, the structure (3) was proposed. This study suggested that enzymes participating



(3)

[90] K. Framberg, H. Mayer, J. Weckesser, and G. Drews, *Arch. Mikrobiol.*, 1974, **98**, 239.
[91] H. Mayer, K. Framberg, and J. Weckesser, *European J. Biochem.*, 1974, **44**, 181.
[92] J. Weckesser, G. Drews, J. Roppel, H. Mayer, and I. Fromme, *Arch. Mikrobiol.*, 1974, **101**, 233.
[93] C. Barber and E. Eylan, *Microbios*, 1974, **9**, 173.
[94] C. G. Hellerqvist, U. Rudén, and P. H. Mäkelä, *European J. Biochem.*, 1972, **25**, 96.

in lipopolysaccharide synthesis act independently of each other, provided that they are offered acceptable substrates formed in previous stages of biosynthesis. Other hybrid strains obtained using modification genes from group B have been studied.[95] Analysis of the lipopolysaccharides of the new hybrids revealed that all the expected *O*-acetylations and D-glucosylations, determined by either B- or C_2-group genes or enzymes, were present. Cross-agglutination and absorption experiments have shown that there are common antigenic determinants between *Salmonella cholerae*, *S. aberdeen*, and *Metschnikowia pulcherrima*.[96] O-Antigen mutants have been obtained from *S. durban*, a group D organism.[97] Serological investigation demonstrated that the mutants lost the O-9 antigen factor of the parent organism, but acquired the O-2 factor specific to group A Salmonella. Lipopolysaccharides of the mutant strains contained paratose, which determines the specificity of the O-2 factor, but tyvelose, present in the wild-type polymer, was not found in the mutants. These findings indicate that species of group A Salmonella may be derived from group D organisms. A mutant strain of *S. durban* possessing the O-2 antigen and twelve mutants of group A Salmonella have been shown to be defective in CDP-paratose-2-epimerase activity.[98] Comparative chemical analyses have been performed on the lipopolysaccharides of a Salmonella T2 form and a $T2^-$ mutant derived from it.[99] The results showed that the T2-specific structure is represented by a substituted 2-acetamido-2-deoxy-D-glucosyl residue, which is attached to the core at the same position as the $T1^-$- and O-specific structures in the corresponding lipopolysaccharides. Another report has described the isolation and properties of 12_2^- mutants of stable 12_2^+ strains from group B Salmonella; the factor 12 is responsible for the D-glucosylation of lipopolysaccharides at O-4 of D-galactosyl residues in the O side-chains.[100] Enzymic reactions involved in the formation of the O-34 antigen of group E_3 Salmonella-carrying phages ε^{15} and ε^{34} have been studied *in vitro* by the use of cells defective in UDP-D-glucose epimerase activity.[101] Incorporation of D-glucose from UDP-D-glucose into the O-34 antigen required polymerization of the D-mannosyl-L-rhamnosyl-D-galactose repeating units, which were then able to accept D-glucosyl residues. The product incorporating D-glucose was identified as the haptenic O-polysaccharide. It was proposed that lipid intermediates are formed by reactions (1) and (2).

$$\text{UDP-D-glucose} + \text{lipid} \rightleftarrows \text{D-glucosyl-lipid} + \text{UDP} \quad (1)$$

$$n\ \text{D-glucosyl-lipid} + (\text{mannosylrhamnosylgalactosyl})_n\text{-lipid} \rightarrow [\text{mannosylrhamnosyl(glucosyl)galactosyl}]_n\text{-lipid} + n\ \text{lipid} \quad (2)$$

The enzymic activity catalysing reaction (1) was found in all cells carrying phage ε^{34} and also in a mutant phage, $\varepsilon^{34}_{O34\text{-}1}$, defective in the formation of O-34 antigen. The catalytic activity needed to carry out reaction 2 was found only in cells

[95] U. Rudén and P. H. Mäkelä, *European J. Biochem.*, 1974, **48**, 11.
[96] N. Aksoycan and H. Daglioglu, *Ann. Microbiol.*, 1973, **124B**, 115.
[97] T. Uchida, T. Matsumoto, and T. Sasaki, *J. Bacteriol.*, 1974, **117**, 8.
[98] T. Sasaki and T. Uchida, *J. Bacteriol.*, 1974, **117**, 13.
[99] M. Bruneteau, W. A. Wolk, P. P. Singh, and O. Lüderitz, *European J. Biochem.*, 1974, **43**, 501.
[100] P. H. Mäkelä, *J. Bacteriol.*, 1973, **116**, 847.
[101] T. Sasaki, T. Uchida, and K. Kurahashi, *J. Biol. Chem.*, 1974, **249**, 761.

carrying both phages ε^{15} and ε^{34}, but not in cells with ε^{15} and the mutant phages. Degradative studies performed on either the original or the dephosphorylated core oligosaccharides derived from lipopolysaccharides of Salmonella mutants have revealed the presence of a branched heptose trisaccharide, *viz.* [Hep III-(1 → 7)]-Hep II-(1 → 3)-Hep I.[102] The heptose II unit was also found to be substituted at either positions 2, 4, or 6 by a phosphate group, and the degree of substitution of the main chain with heptose III varied from 20 to 90% in different lipopolysaccharides. From this and previous studies, the structure of the inner core region can be represented as (4).

D-Glc-(1 → 3)-Hep II-(1 → 3)-Hep I-(1 → 5)-KDO
7 4
↑ ↑
1 1
Hep III PP-ethanolamine

KDO is 3-deoxy-2-octulosonic acid

(4)

Brief treatment of *S. enteritidis* cells with H_4edta resulted in the release of an aggregate of high molecular weight, which, under selected conditions, could be fractionated into several components possessing different molecular weights and properties.[103] The aggregate, composed of 50% protein, 40% lipopolysaccharide, and 10% phospholipid, was released from the cells with minimal contamination by internal constituents; except for an increased lag-period of growth, no decrease in viability or lysis could be demonstrated in cells treated in this manner. The compositions of lipid-A and core oligosaccharides of *S. friedenau* and *S. typhimurium* were found to be independent of the conditions of aeration during growth.[104] Similarly, the molar ratios of sugars in the O-specific polysaccharide chains remained constant, although the number of repeating units constituting the chains in *S. typhimurium* varied. These results have further suggested that, irrespective of the conditions of aeration, the R-core oligosaccharides of both Salmonella strains contain only two residues of 3-deoxy-2-octulosonic acid and that not every repeating unit contains a factor 12_2 (*S. typhimurium*) or a factor 1 (*S. friedenau*). Serological investigations showed that the unbound core structures of chemotype Ra are present in the lipopolysaccharide from *S. friedenau.*

The repeating tetrasaccharide structure of the O-specific antigen of *S. illinois* has been shown to be (5).[105] When this tetrasaccharide reacted with *o*-phenylenediamine and *m*-nitrophenylhydrazine and the product was reduced, a 1-(3-aminophenyl)flavazole was obtained, and this could be coupled to edestin.

α-D-Glc
1
↓
4
-β-D-Gal-(1 → 6)-α-D-Man-(1 → 4)-L-Rha-

(5)

[102] G. Hämmerling, V. Lehmann, and O. Lüderitz, *European J. Biochem.*, 1973, **38**, 453.
[103] J. R. Chipley, *Microbios*, 1974, **10**, 139.
[104] I. Fromme and S. Schlect, *Z. Bakteriol. Parasitenkd. Infekt. Hyg. I*, 1973, **224**, 331.
[105] G. Kleinhammer, K. Himmelspach, and O. Westphal, *European J. Immunol.*, 1973, **3**, 834.

Antisera obtained after immunization of rabbits with the flavazole–edestin complex produced antibodies directed predominantly against the non-reducing group α-D-Glc-(1 → 4)-β-D-Gal-(1 → 6)-D-Man (O-factor 34) and only to a slight extent against the internal grouping. Thus, by immunization with a flavazole–edestin conjugate, a largely factor-specific antiserum (anti-34) was obtained. Three antigenic determinants (40a, 40b, and 40c) have been found on the polysaccharide R^- of the wild-type strain of *S. johannesburg*.[106] The immunodominant sugars were shown to be 2-acetamido-2-deoxy-D-galactose for 40a and 40b, and 2-acetamido-2-deoxy-D-glucose for 40c. Inhibition studies with oligosaccharides isolated from the polysaccharide R^- suggested that 40a is the hexasaccharide (6).

→ 4)-[D-Gal*N*Ac-(1 → 6)]-D-Gal*N*Ac-(1 → 3)-D-Man-(1 → 4)-D-Glc-(1 → 4)-

[D-Gal*N*Ac-(1 → 6)]-D-Gal*N*Ac

(6)

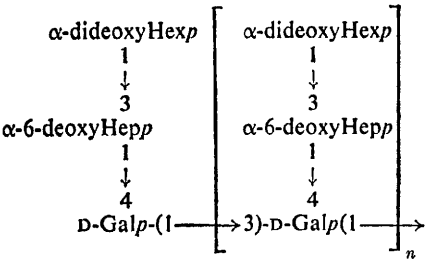
A series of rough *S. minnesota* mutants, with mutations leading to various defects in the biosynthesis of the cell-wall lipopolysaccharides, have been analysed for their common antigen.[107] It was found that the core structures are not necessarily part of the antigen, but that all *rfe*$^-$ mutants, which had a complete lipopolysaccharide core but which were defective in the synthesis of O-side-chains, were also defective in the synthesis of the common antigen. Lipopolysaccharides from *S. newington* have been fractionated by ion-exchange chromatography to yield products showing differences in the number of repeating units in the O-antigen side-chains and in the extent of substitution with ester phosphate.[108] Further fractionation was achieved after removal of lipid A from each of the fractions, indicating a high degree of structural heterogeneity in the original lipopolysaccharide. The lipopolysaccharide in the outer membrane of *S. typhimurium* has been calculated to cover 15—25% of the surface of the bacterium at a density of 0.7—1.0 × 10^5 molecules per square μm.[109] A virulent *S. typhimurium* strain and mutants derived from it with deficiencies in the cell-wall lipopolysaccharides have been examined for sensitivity to various bactericidal systems operating within the polymorphonuclear leucocytes.[110] The results demonstrated the decisive influence of the surface structure of the bacterial cell on the efficiency of intracellular bactericidal systems. The isolation, phage sensitivity, sugar composition, serological characteristics, and genetics of several mutants of *S. typhimurium*, which had lesions in the heptose region of the lipopolysaccharide, have been reported.[111] These mutants were more sensitive to the growth-inhibitory effects of some antibiotics and of deoxycholate than were smooth strains, but were less sensitive to other antibiotics.[112] Coupled with the results of studies using lysozyme and H_4edta, it was concluded that, since the

[106] R. Girard and A. M. Staub, *Carbohydrate Res.*, 1974, **37**, 127.
[107] P. H. Mäkelä, H. Mayer, H. Y. Whang, and E. Neter, *J. Bacteriol.*, 1974, **119**, 760.
[108] J. M. Ryan and H. E. Conrad, *Arch. Biochem. Biophys.*, 1974, **162**, 530.
[109] P. F. Mühlradt, J. Menzel, J. R. Golecki, and V. Speth, *European J. Biochem.*, 1974, **43**, 533.
[110] C. Tagesson and O. Stendahl, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 473.
[111] K. E. Sanderson, J. Van Wyngaarden, O. Lüderitz, and B. A. D. Stocker, *Canad. J. Microbiol.*, 1974, **20**, 1127.
[112] K. E. Sanderson, T. MacAlister, and J. W. Costerton, *Canad. J. Microbiol.*, 1974, **20**, 1135.

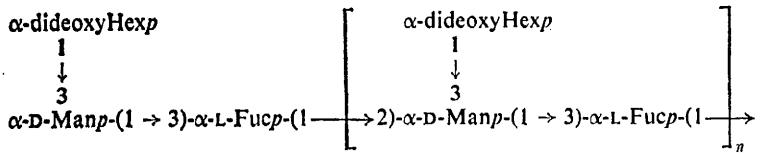
major part of the lipopolysaccharide is in the outer membrane of the cell envelope and since the target of the toxic agents used is located inside this layer, the carbohydrate moiety of the lipopolysaccharide component of the outer membrane is an essential part of a barrier preventing the penetration of large molecules. The oligosaccharide cores from two strains of *Serratia marcescens* have been analysed and shown to contain D-*glycero*-D-*manno*-heptose, L-*glycero*-D-*manno*-heptose, D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and 3-deoxy-2-octulosonic acid, but in different proportions.[113] It was suggested that *S. marcescens* may contain more than one structural type of oligosaccharide core, each core differing in chemical composition from those of other Enterobacteriaceae. Serratigen, an antigenic lipopolysaccharide from *S. marcescens*, was shown by gel-diffusion techniques to be homologous in antigenicity with seramarcide, a polysaccharide obtained from *S. marcescens*.[114] Methylation analysis of the polysaccharide showed it to have a (1 → 3)-galactan structure. To define the immunodominant group of lipid A from the lipopolysaccharide of *Shigella sonnei*, passive haemolysis inhibition studies were performed.[115] 3-Hydroxymyristyl residues attached to 2-amino-2-deoxy-D-glucosyl residues *via* an amide linkage proved to be necessary for lipid-A specificity. Anticomplementary properties of *Sh. sonnei* lipid A were also confirmed. Chemical studies on the *Sh. sonnei* lipid-A portion of the lipopolysaccharide have demonstrated the presence of 2-amino-2-deoxy-β-D-glucosyl-(1 → 6)-2-amino-2-deoxy-D-glucose residues, probably interlinked by pyrophosphate bridges.[116] The amino-sugars of the disaccharide unit are *O*-substituted by fatty-acid (lauryl, 3-D-myristoxymyristyl, 3-D-hydroxymyristyl, and palmityl) residues, and the amino-groups are substituted by 3-D-hydroxymyristyl residues. Rough forms of *Sh. sonnei*, *Sh. boydii*, *Sh. dysenteriae*, and *Sh. flexneri* have been compared, by phage typing and by serological techniques, with known prototypes of Salmonella and *E. coli* R mutants having the Salmonella R_a or *E. coli* R1—R4 lipopolysaccharide core structures, respectively.[117] The classification of the different *Shigella* R mutants as members of distinct core types, according to their respective phage patterns, was fully corroborated by the serological behaviour of the isolated lipopolysaccharides in passive haemagglutination tests. An agglutinable and a non-agglutinable strain of *Vibrio cholerae* were found to have significant differences in the chemical compositions of the respective lipopolysaccharides, although they are composed of the same components.[118] The lipopolysaccharide from the agglutinable strain required prior treatment with alkali to become an effective antigen with sheep cells, probably due to the removal of *O*-acetyl groups. The lipopolysaccharides of *Yersinia enterocolytica* and *Brucella abortus* have been isolated,[119] and the cross-reactivity of the two lipopolysaccharides is thought to be confined to the O-specific side-chains.[120] The chemical and physical properties of the lipopolysaccharide of *Y. pestis* have been reported;[121] D-glucose, D-*glycero*-

[113] C. S. Wang, R. K. Burns, and P. Alaupovic, *J. Bacteriol.*, 1974, **120**, 990.
[114] T. Ikekawa and Y. Ikeda, *Jap. J. Vet. Sci.*, 1973, **35**, 269.
[115] C. Lugowski and E. Romanowska, *European J. Biochem.*, 1974, **48**, 81.
[116] C. Lugowski and E. Romanowska, *European J. Biochem.*, 1974, **48**, 319.
[117] H. Mayer and G. Schmidt, *Z. Bacteriol. Parasitenkd. Infekt. Hyg. I*, 1973, **224**, 345.
[118] B. Guhathakurta and G. C. Dutta, *Appl. Microbiol.*, 1974, **27**, 634.
[119] B. Hurvell, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 105.
[120] B. Hurvell and A. A. Lindberg, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 113.
[121] J. L. Hartley, G. A. Adams, and T. G. Tornabene, *J. Bacteriol.*, 1974, **118**, 848.

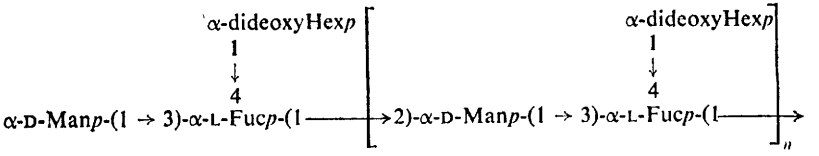
D-*manno*-heptose, L-*glycero*-D-*manno*-heptose, 2-amino-2-deoxy-D-glucose, and 3-deoxy-2-octulosonic acid were found as components, with the lipid-A moiety composed of the amino-sugar substituted with phosphate, amide-linked 3-hydroxymyristate, and amide-bound acetate. The sugar compositions of the lipopolysaccharides of several strains of *Y. pseudotuberculosis* have been reported.[122] In addition to all strains containing D-glucose, 3-deoxy-2-octulosonic acid, L-*glycero*-D-*manno*-heptose, D-*glycero*-D-*manno*-heptose, and amino-sugars, the presence of 6-deoxy-D-*manno*-heptose was reported. Lipopolysaccharides or lipid-free polysaccharides of all of the ten known serogroups and subgroups of *Y. pseudotuberculosis* have been subjected to methylation and hydrolysis with acid; the methylated components were determined as the alditol acetates by g.l.c.–m.s.[123] The results indicated that the O-specific side-chains of nine of the serotypes are composed of oligosaccharide repeating units in the form of four alternative general structures (7)—(10) in which a terminal 3,6-



(7) Hep = D-*manno*-heptose



(8)



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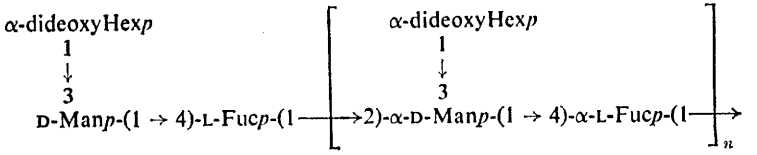


(10)

[122] G. Seltmann, W. Beer, and E. Thal, *Z. allgem. Mikrobiol.*, 1974, **14**, 73.
[123] K. Samuelson, B. Lindberg, and R. R. Brubaker, *J. Bacteriol.*, 1974, **117**, 1010.

dideoxyhexose residue can vary. 6-Deoxy-D-*manno*-heptose [see (7)] has not previously been reported to occur naturally. By contrast, O-groups in the lipopolysaccharide of a new serotype were found to contain a 3,6-dideoxyhexose (colitose) and 2-acetamido-2-deoxy-D-galactose.

The role of membranes in the biosynthesis of lipopolysaccharides has been reviewed.[124]

Lipid A of a lipopolysaccharide from a Gram-negative marine bacterium has been shown to contain D-glucose, D-galactose, and amino-sugars, but neither heptose nor 3-deoxy-2-octulosonic acid, indicating that the preparation did not contain the backbone of the lipopolysaccharide.[125] A new class of lipopolysaccharide has been isolated from *Thermoplasma acidophilum*.[126] Extraction of the cells with hot phenol solubilized a material (mol. wt. 1.2×10^6) composed of 20% glycerol diether and 80% carbohydrate (D-mannose : D-glucose, 24 : 1), in which most of the D-mannosyl residues were susceptible to α-mannosidase. The alkyl glycerol terminus is a 1,2-glycerol diether composed of forty-carbon isopranol side-chains. The blue-green alga *Anabaena variabilis* contains an O-antigenic lipopolysaccharide, which is located in the outermost cell-wall layer; the polysaccharide moiety is composed of residues of L-rhamnose, 3-*O*-methyl-L-rhamnose (acofriose), D-mannose, D-glucose, D-galactose, and 2-amino-2-deoxy-D-glucose.[127]

A new method has been developed for the specific degradation of methylated polysaccharides containing a number of unsubstituted hydroxy-groups at defined positions.[128] Oxidation and subsequent β-elimination provided evidence concerning the sequence of sugar residues in the original polysaccharide. The accuracy of this method was demonstrated from studies of lipopolysaccharides and capsular polysaccharides of known structure.

Capsular Polysaccharides.—The capsular polysaccharide of *Clostridium perfringens* has been reported to have a molecular weight of 4×10^4, which rises to 1.2×10^5 on aggregation; it is composed of D-glucose, D-galactose, 2-amino-2-deoxy-D-galactose, and iduronic acid (4 : 5 : 1.7 : 1).[129] Extracellular polysaccharides of the genus *Bacillus* have been shown to cross-react with the capsular polysaccharides of *Diplococcus pneumoniae* type III, *Haemophilus influenzae* type b, and *Neisseria meningitidis* group A.[130] A passive hemagglutination test, using erythrocytes coated with acidic polysaccharides, has been used to determine antibodies against type-specific capsular polysaccharides in the *Klebsiella* group.[131] Methylation analysis of and partial acidic hydrolysis studies on the *Klebsiella* K7 capsular polysaccharide and its carboxy-reduced derivative have indicated the occurrence of D-glucopyranosyluronic acid, D-mannopyranosyl, and D-glucopyranosyl residues.[132] D-Galactopyranosyl and pyruvic acid residues were

[124] S. Kanegasaki, *Seikagaku*, 1974, **46**, 297.
[125] A. Mongillo, K. Deloge, D. Pereira, and G. P. O'Leary, *J. Bacteriol.*, 1974, **117**, 327.
[126] K. J. Mayberry-Carson, T. A. Langworthy, W. R. Mayberry, and P. F. Smith, *Biochim. Biophys. Acta*, 1974, **360**, 217.
[127] J. Weckesser, A. Katz, G. Drews, H. Mayer, and I. Fromme, *J. Bacteriol.*, 1974, **120**, 672.
[128] L. Kenne, J. Lönngren, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 3612.
[129] L. Lee and R. Cherniak, *Infection and Immunity*, 1974, **9**, 318.
[130] R. L. Myerowitz, R. E. Gordon, and J. B. Robbins, *Infection and Immunity*, 1973, **8**, 896.
[131] J. Eriksen, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 309.
[132] G. G. S. Dutton, A. M. Stephen, and S. C. Churms, *Carbohydrate Res.*, 1974, **38**, 225.

found to be linked to the main chain on the D-mannosyl residues at O-3 and on the D-glucosyl residues at O-4 and O-6, respectively; the indications are that nine sugar residues and pyruvic acid constitute a repeating unit. The sequence → 3)-β-D-GlcUA*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Glc*p* was demonstrated by the isolation from the polysaccharide of an aldotetraouronic acid of this structure. Gel-permeation chromatography has been used to determine the molecular-weight distribution of the products at various stages of acid hydrolysis of several *Klebsiella* capsular polysaccharides.[133] Structurally significant oligosaccharides, which are believed to correspond closely to the chemical repeating units in the polysaccharide, were detected, together with aggregates having higher molecular weights, thus providing supporting evidence for the view that relatively simple sequences of sugars are repeated throughout the entire molecular structure of these polysaccharides. *Klebsiella* type 52 polysaccharide has been investigated and was shown to be composed of D-glucuronic acid, D-galactose, and L-rhamnose (1 : 3 : 2).[134] Oligosaccharides isolated after hydrolysis of the methylated polysaccharide with acid were analysed by g.l.c.–m.s., and the methylated polysaccharide was also specifically degraded by successive treatments with base and acid, followed by characterization of the products. The structure of the hexasaccharide repeating unit (11) was deduced, although the anomeric natures of the sugar residues remain to be determined.

→ 3)-D-Gal*p*-(1 → 2)-L-Rha*p*-(1 → 4)-D-GlcUA*p*-(1 → 3)-D-Gal*p*-(1 → 4)-L-Rha*p*-(1 →
2
↑
1
D-Gal*p*

(11)

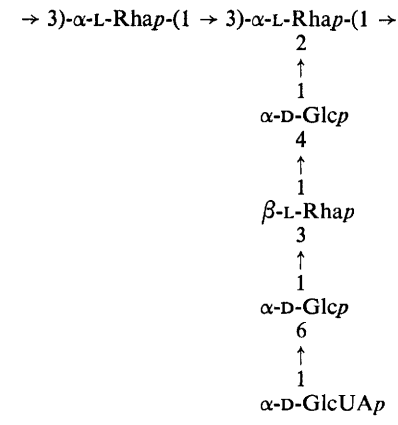
The capsular polysaccharide from *K. pneumoniae* type 1 has been fractionated into an acidic component and a neutral component, using cetylpyridinium chloride.[135] The neutral component exhibited an extremely strong adjuvant effect. It was shown that the substance active towards the neutral polysaccharide was homogeneous by gel-permeation chromatography and ultracentrifugation, and contained hexose, hexuronic acid, amino-sugars, protein, and lipids. When the capsule of *K. pneumoniae* type 1 and the slime of *Enterobacter aerogenes* were examined by electron microscopy, using the freeze-etch technique, the capsule was found to be composed of several layers of polysaccharide, whereas the slime was shown to be composed of a diffuse network of fibrils.[136] When the polysaccharide from the slime was purified and then freeze-etched, it resembled the layered structure of the capsule of *K. pneumoniae*. It was suggested that either the charge or the dielectric constant of the slime polysaccharide was altered during purification, thereby permitting layering to occur. Of three different techniques used for detecting polysaccharide antigens of meningococcal species,

[133] S. C. Churms and A. M. Stephen, *Carbohydrate Res.*, 1974, **35**, 73.
[134] H. Björndal, B. Lindberg, J. Lönngren, M. Mészáros, J. L. Thompson, and W. Nimmich, *Carbohydrate Res.*, 1973, **31**, 93.
[135] I. Nakashima and N. Kato, *Jap. J. Microbiol.*, 1973, **17**, 461.
[136] E. L. Springer and I. L. Roth, *Arch. Mikrobiol.*, 1973, **93**, 277.

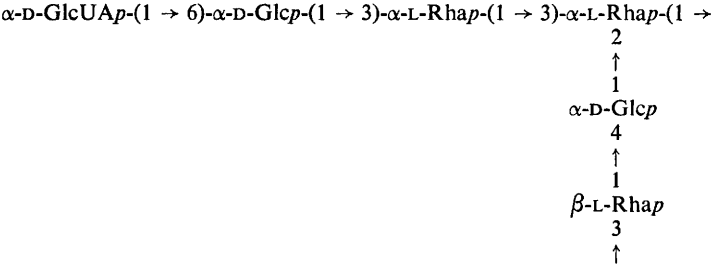
immunoelectrophoresis was shown to be the most sensitive, and it was considered for a possible diagnostic procedure.[137] Hemagglutinating and precipitating antibodies to a subgroup determinant on *Neisseria meningitidis* group C capsular polysaccharide were detected in four antisera developed against isolated group C antigens.[138] Evidence from enzymic and oxidative studies indicated that the determinant is carbohydrate-dependent and may confer resistance to neuraminidase on group C polysaccharides containing it. The basic structure of *N. meningitidis* serogroup A has been studied by ^{13}C n.m.r. spectroscopy, which confirmed it to be an essentially (1 → 6)-linked homopolymer composed of partially *O*-acetylated 2-amino-2-deoxy-D-mannosyl phosphate residues.[139] Previous studies on the related serogroup X polysaccharide had indicated it to be a homopolymer of either (1 → 3)- or (1 → 4)-linked, *O*-acetylated 2-acetamido-2-deoxy-D-glucosyl phosphate residues. By use of ^{13}C n.m.r. spectroscopy, it was demonstrated that the serogroup A polysaccharide contains both mono- and non-acetylated residues of 2-acetamido-2-deoxy-D-mannose in the ratio of 7 : 3, with the acetyl substituents located at O-3 of these residues. Under conditions of mild acidic hydrolysis, the serogroup X polysaccharide yielded only 2-acetamido-2-deoxy-D-glucose 4-phosphate, indicating that adjacent sugar units are (1 → 4)-linked by phosphoric diester bonds.[140] Other studies on the polymer indicated it to be linear, with an average chain length ($\overline{CL}$) of fifty units. Serological studies on the polysaccharide showed that its antiserum is highly specific for the position of the phosphoric diester linkage, and that the main serological specificity is directed towards oligomer units. A simple gel-diffusion technique, when evaluated against a quantitative precipitin test, gave close correlation in the assay of pneumococcal capsular polysaccharides.[141] The physical and immunological properties of a type-specific, pneumococcal capsular polysaccharide produced during infection have been recorded.[142] Double-diffusion studies on pneumococcal capsular polysaccharides prepared by conventional methods revealed the presence of a contaminating C-polysaccharide, which could be removed by electrophoresis.[143] Further information on the structure of *Diplococcus pneumoniae* type II capsular polysaccharide has been reported; oxidation with chromium trioxide of the acetylated polysaccharide, followed by reduction and methylation analysis, established the presence of a β-L-rhamnosyl residue linked to O-4 of an α-D-glucosyl residue.[144] Together with the results of previous studies, it was suggested that either (12) or (13) should represent the actual structure of the repeating unit.

D. pneumoniae type III polysaccharide has been reported to stimulate the synthesis of DNA in non-sensitized spleen cells cultured *in vitro*.[145] It was concluded that the polysaccharide is a B-cell mitogen capable of directly activating DNA and antibody syntheses in these cells. Comparison of the inhibitory

[137] J. Casal, P. Pérez-Breña, and C. Martín-Bourgon, *Microbiol. Esp.*, 1973, **26**, 85.
[138] M. A. Apicella, *J. Infectious Diseases*, 1974, **129**, 147.
[139] D. R. Bundle, I. C. P. Smith, and H. J. Jennings, *J. Biol. Chem.*, 1974, **249**, 2275.
[140] D. R. Bundle, H. J. Jennings, and C. P. Kenny, *J. Biol. Chem.*, 1974, **249**, 4797.
[141] R. K. Porschen and E. R. Kennedy, *J. Immunol. Methods*, 1974, **4**, 107.
[142] J. D. Coonrod, *J. Immunol.*, 1974, **112**, 2193.
[143] K. A. Broholm and J. Holmgren, *Acta Path. Microbiol. Scand.*, 1973, **81B**, 321.
[144] O. Larm, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1973, **31**, 120.
[145] A. Coutinho and G. Möller, *European J. Immunol.*, 1973, **3**, 608.



(12)



(13)

powers of 3-*O*-β-D-galactopyranosyl-L-rhamnose, 4-*O*-β-D-galactopyranosyl-L-rhamnose, and 3-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-rhamnose with those of a selection of other disaccharides on the precipitin reaction between type VII anti-pneumococcal horse serum and either type VII pneumococcal polysaccharide or Tamarind A polysaccharide showed that *O*-D-galactosyl and *O*-(2-acetamido-2-deoxy-D-glucosyl)-L-rhamnosyl groups are important serological determinants in the pneumococcal type VII polysaccharide.[146] Structural studies have been reported on the capsular polysaccharide of *D. pneumoniae* type XII, which was shown to be composed of D-glucose and D-galactose (2 : 1), 2-acetamido-2-deoxy-L-fucose, and 2-acetamido-2-deoxy-D-galactose.[147] From the results of methylation analysis and periodate oxidation, it was concluded that the polysaccharide consists of a hexosamine backbone that is substituted by D-galactosyl and kojibiosyl side-chains. The proposed terminal D-galactosyl residues apparently are sterically hindered from interacting with several D-galactose-binding proteins. Kojibiose has been isolated from a partial acidic hydrolysate of *D. pneumoniae* type XII capsular polysaccharide (S XII).[148] A precipitation curve was generated between concanavalin A and S XII, but this

[146] R. R. King and C. T. Bishop, *Carbohydrate Res.*, 1974, **32**, 239.
[147] J. L. Duke, I. J. Goldstein, and J. A. Cifonelli, *Carbohydrate Res.*, 1974, **37**, 81.
[148] I. J. Goldstein, J. A. Cifonelli, and J. Duke, *Biochemistry*, 1974, **13**, 867.

line was eliminated following periodate oxidation and borohydride reduction of S XII, owing to the destruction of the kojibiosyl residues. Since kojibiose was shown to be a strong inhibitor of the reaction with concanavalin A, it seems likely that the disaccharide is responsible for the interaction of S XII with the lectin. The membrane teichoic acid of *S. faecalis* has been found to precipitate in equine and rabbit anti-pneumococcal type XII sera.[23] This teichoic acid has been shown to contain kojibiosyl residues, and the reaction confirmed the presence of like residues in the capsular polysaccharide. Extraction of *Staphylococcus aureus* M with trichloroacetic acid solubilized a capsule composed of taurine and an aminogalacturonic acid, thereby distinguishing it chemically from all known staphylococcal capsules.[149]

Extracellular and Intracellular Polysaccharides.—The exopolysaccharides of a number of slime-producing *Achromobacter* spp. are composed of residues of D-glucose, D-galactose, pyruvic acid, and acetic acid (2 : 2 : 2 : 1).[150] From the results of methylation analysis and partial acid hydrolysis, each polysaccharide appears to contain an unbranched chain of alternate residues of (1 → 3)-linked β-D-galactose and β-D-glucose, with the pyruvate linked by acetal bonds at O-4 and O-6 of the D-galactosyl residues. A polysaccharide produced by *Bacillus subtilis* FT3 has a molecular weight of 2.5—3.5 × 10^4 and contains D-glucose, D-galactose, L-fucose, D-glucuronic acid, and *O*-acetyl groups.[151] The polysaccharide exudate of *Erwinia amylovora*, after ion-exchange chromatography, was shown to be a single heteropolymer containing residues of D-mannose, D-galactose, D-glucose, and uronic acid.[152] The accumulation of an intracellular glucan early in the sporulation of *B. cereus* and its degradation at the time of maturation of the spores has led to the suggestion that the polysaccharide may serve as sources of carbon and energy for sporulation.[153] The iodine-absorption spectrum indicated that this α-D-glucan has a degree of branching intermediate between those of glycogen and amylopectin. Glycogen synthesized by the purple sulphur bacterium *Chromatium*, strain D, has been characterized by degradation with α- and β-amylases and pullulanase.[154] From these and other studies, it was shown to have a $\overline{CL}$ of 11, an internal $\overline{CL}$ of 3, and an exterior $\overline{CL}$ of 7. An iodophilic, intracellular polysaccharide from *Clostridium pasteurianum* has been shown to be a linear molecule containing α-(1 → 4)- and α-(1 → 6)-linked D-glucopyranosyl residues in the ratio of 3 : 2.[155] Although the rate of biosynthesis of the glucan of *C. pasteurianum* is probably reflected by the energy status of the adenylate pool of the organism, it is not subject to the fine control effected by the allosteric activity of ADP-D-glucose pyrophosphorylase that is a feature of the production of glycogen in many bacteria.[156] Although the nature of its genetic lesion was unknown, a mutant strain of *C. pasteurianum* was found to be deficient in glucan phosphorylase activity, and was con-

[149] D. F. Liau, M. A. Melly, and J. H. Nash, *J. Bacteriol.*, 1974, **119**, 913.
[150] L. P. T. M. Zevenhuizen and A. G. Ebbink, *Arch. Mikrobiol.*, 1974, **96**, 75.
[151] N. Morita and S. Murao, *J. Ferm. Technol.*, 1974, **52**, 438.
[152] S. J. Eden-Green and M. Knee, *J. Gen. Microbiol.*, 1974, **81**, 509.
[153] J. A. Slock and D. P. Stahly, *J. Bacteriol.*, 1974, **120**, 399.
[154] F. Hara, T. Akazawa, and K. Kojima, *Plant Cell Physiol.*, 1973, **14**, 737.
[155] E. J. Laishley, R. G. Brown, and M. C. Otto, *Canad. J. Microbiol.*, 1974, **20**, 559.
[156] R. L. Robson, R. M. Robson, and J. G. Morris, *Biochem. J.*, 1972, **337**, 4P.

sequently prevented from making use of its glucan reserves, although these still accumulated in the usual way.[157] The *de novo* synthesis of bacterial glycogen by ADP-D-glucose:α-glucan 4-glucosyl transferase in *Escherichia coli* has been reviewed.[158] A mutant of *E. coli* was able to synthesize glycogen when grown on D-gluconate; the cells contained all the enzymes for glycogen synthesis, but were devoid of glucoside primer.[159] However, this strain was able to synthesize glycogen rapidly when it was supplemented with D-glucose. This *de novo* synthesis does not seem to be limited by the energy charge of the cells. Another type of mutant has been isolated from *E. coli* with a deficiency in the branching-enzyme activity, causing an accumulation of a linear polysaccharide similar to amylose.[160] Amylase deficiency and an altered ADP-D-glucose pyrophosphorylase activity are considered to be important in the accumulation of amylose and its aggregation. An inverse relationship between cell growth and glycogen deposition has been observed intracellularly in *Fusiformis necrophorus*.[161]

The results of methylation analysis of a dextran produced by *Leuconostoc mesenteroides* NRRL B-1397 have indicated that branching occurs mainly at O-2, and the remainder at O-3, on a (1 → 6)-linked backbone.[162] A modified dextran, in which some of the non-reducing D-glucosyl residues had been converted into D-glucuronic acid residues by catalytic oxidation, yielded, after acidic hydrolysis, 2-*O*-(α-D-glucopyranosyluronic acid)-D-glucose (as the major product), 6-*O*-(α-D-glucopyranosyluronic acid)-D-glucose, and mixtures of aldotri-, aldotetra-, and aldopenta-ouronic acids containing both (1 → 6)- and (1 → 2)-linked D-glucosyl residues. It was concluded that the branches at O-2 are mainly single D-glucosyl residues, whereas those at O-3 may be longer than two D-glucosyl residues, forming a highly branched structure having an average repeating unit of five sugar residues. Methylation analysis of five fractions of a dextran elaborated by *L. mesenteroides* NRRL B-1299 has shown that each fraction is a highly branched dextran, with the branches joined mainly through O-2 and, possibly, a small number of D-glucosyl residues branched both at O-2 and O-3.[163] The solubility characteristics of the polysaccharide in borate buffers were attributed to the content of linear α-(1 → 3)-D-glucosyl residues. In dextran *S* elaborated by *L. mesenteroides*, the polymer chain has been shown to consist principally of segments of isomaltose homologues, which are mutually linked through positions 1 and 2 of their terminal D-glucosyl residues. The average repeating unit [*e.g.* (14)] contains a total of fifteen D-glucosyl residues and possesses five branch points.[164] The branches consist mainly of α-D-glucosyl groups, and some appear to be terminated by α-nigerosyl groups. Synthesis of an insoluble dextran by the soluble sucrase of *Streptococcus mutans* has given rise to two forms of dextransucrase bound to the insoluble polysaccharide, *viz.* a reversibly

[157] B. M. Mackey and J. G. Morris, *F.E.B.S. Letters*, 1974, **48**, 64.
[158] J. Fox, L. D. Kennedy, J. S. Hawker, J. L. Ozbun, E. Greenberg, C. Lammel, and J. Preiss, *Ann. New York Acad. Sci.*, 1973, **210**, 90.
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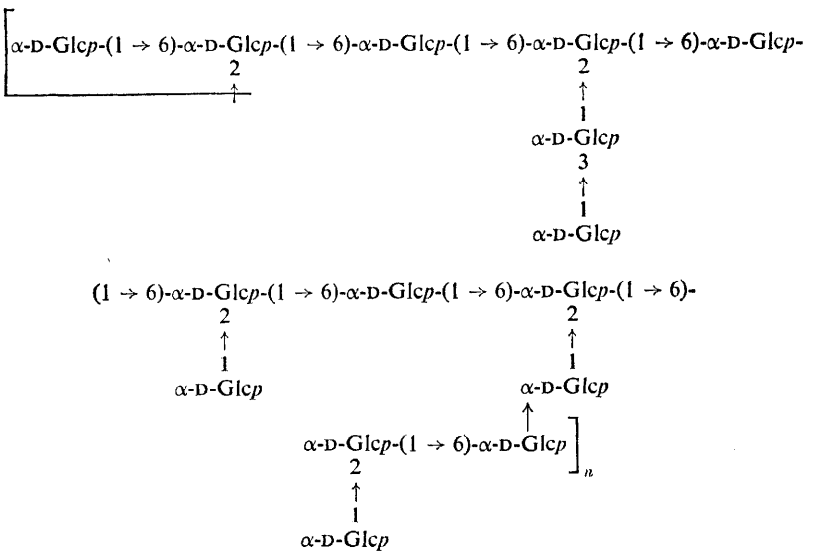
[160] C. Lares, C. Frixon, N. Creuzet-Sigal, and P. Thomas, *J. Gen. Microbiol.*, 1974, **82**, 279.
[161] A. Wahren, *Acta Path. Microbiol. Scand.*, 1974, **82B**, 635.
[162] H. Miyaji, A. Misaki, and M. Torii, *Carbohydrate Res.*, 1973, **31**, 277.
[163] M. Kobayashi, K. Shishido, T. Kikuchi, and K. Matsuda, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2763.
[164] E. J. Bourne, R. L. Sidebotham, and H. Weigel, *Carbohydrate Res.*, 1974, **34**, 279.



(14)

bound enzyme, which could be eluted in solutions of clinical dextran, and an irreversibly bound enzyme.[165] During the synthesis of insoluble dextran, dextransucrase was progressively converted from the soluble form into the reversibly bound form and then into the irreversibly bound form; finally, it was inactivated as the insoluble polysaccharide accumulated.

The results of methylation analysis, Smith degradation, and enzymic studies have furnished unambiguous evidence of an α-(1 → 3)-linked backbone in the insoluble glucan of a cariogenic *S. mutans*, and it was clear that most (1 → 6)-linked D-glucosyl residues are located as side-chains, forming a ramified, comb-like structure.[166] Taking into account its insolubility characteristics, the native D-glucan appears to contain relatively long, α-(1 → 3)-linked, linear sequences in parts of the molecule. It was not possible to say whether or not the main chain consists entirely of α-(1 → 3)-linkages or contains also α-(1 → 6)-linkages, but the finding of a small proportion of glycerol in the hydrolysate of the Smith-degraded D-glucan suggests the possibility that long chains of α-(1 → 3)-linked D-glucosyl residues are flanked by α-(1 → 6)-linkages. The extracellular polysaccharides produced from sucrose by D-glucosyltransferases present in the culture filtrate of a cariogenic *S. mutans* have been isolated.[167] The polymers have been characterized as a water-insoluble glucan containing both α-(1 → 3)- and α-(1 → 6)-linkages, a water-soluble glucan comprising mainly α-(1 → 6)-linkages, and a water-soluble fructan containing β-(2 → 1)-linkages. From competitive inhibition of the agglutination of *S. mutans* by *Leuconostoc* dextran, it was suggested that the spatial arrangements of the groups at C-3 and C-1 are

[165] M. M. McCabe and E. E. Smith, *Infection and Immunity*, 1973, **7**, 829.
[166] S. Ebisu, A. Misaki, K. Kato, and S. Kotani, *Carbohydrate Res.*, 1974, **38**, 374.
[167] J. K. Baird, V. M. C. Longyear, and D. C. Ellwood, *Microbios*, 1973, **8**, 143.

important in the dextran.[168] Periodate oxidation of the dextran, with or without subsequent borohydride reduction, prevented the agglutination with *S. mutans*. From the results of treatment of the bacteria with proteolytic enzymes, the dextran receptor-site was indicated to be a protein. The influence of fluoride ions on the levels of extracellular polysaccharides in dental microbial plaques was only observed at concentrations of 10 p.p.m. or more.[169] Concanavalin A has been shown to inhibit the hydrolysis of dextrans and glycogen by α-glucosidase, but it had no effect on the enzymic hydrolyses of amylose and maltose.[170] The degree of inhibition depended on the ratio of lectin to dextran and on the structure of the dextran. Dextran has been shown to adsorb to normal human erythrocytes from simple salt solutions, the amount adsorbed varying linearly with the concentration of dextran.[171] The relative zeta-potential (Z) of erythrocyte cells in dextran solutions depended on the species of univalent counter-ion present, but not on the co-ion. The data are consistent with a model in which intracellular electrostatic repulsions can be sufficiently enhanced by the presence of adsorbed dextran to overcome the tendency of polymer bridging to aggregate the cells.[172] In a study of the properties of the water–gelatin–dextran system, the self-association of gelatin was shown to be the main reason for its incompatibility with dextran.[173] At low concentrations of dextran, the gelatinous system is monophasic, but proceeds to a two-phase state at high concentrations.

A homogeneous reaction system for the dansylation of proteins has been developed.[174] Enhancement of the fluorescence intensity of the dansylated amino-acids by addition of cyclohepta-amylose was attributed to the inclusion of a dansyl group in the hydrophobic cavity of the molecule. The rates of release of substituted phenols from diaryl carbonates and diaryl methylphosphonates were accelerated in the presence of cycloamyloses.[175] Crystallographic and stoicheiometric data have been obtained for several inclusion complexes of cyclohexa-amylose.[176] The cell dimensions and space-group symmetries reflect the packing arrangement of the torus-shaped host molecules and are largely determined by the size and ionic character of the guest molecules. The structure of the iodine–cyclohexa-amylose tetrahydrate complex has been solved by heavy-atom techniques.[177] The molecules were found to be arranged in herring-bone 'cage-type' fashion, with four water molecules as space-filling mediators; the structure is held together by an intricate network of hydrogen bonds. Some aspects of the thermodynamics of the binding of guest molecules to cyclohexa- and cyclohepta-amyloses have been reported.[178] Although it was shown that the two cyclodextrins have similar equilibrium constants for binding the same

[168] J. Kelstrup and T. D. Funder-Neilsen, *J. Gen. Microbiol.*, 1974, **81**, 485.
[169] Z. Broukal and O. Zajiček, *Caries Res.*, 1974, **8**, 97.
[170] M. E. Preobrazhenskaya, *Biokhimiya*, 1973, **38**, 763.
[171] D. E. Brooks, *J. Colloid Interface Sci.*, 1973, **43**, 700.
[172] D. E. Brooks, *J. Colloid Interface Sci.*, 1973, **43**, 714.
[173] V. B. Tolstogusov, V. P. Belkina, V. J. Gulov, V. J. Grinberg, E. F. Titova, and E. M. Belavzeva, *Stärke*, 1974, **26**, 130.
[174] T. Kinoshita, F. Iinuma, and A. Tsuji, *Analyt. Biochem.*, 1974, **61**, 632.
[175] H. J. Brass and M. L. Bender, *J. Amer. Chem. Soc.*, 1973, **95**, 5391.
[176] R. K. McMullan, W. Saenger, J. Fayos, and D. Mootz, *Carbohydrate Res.*, 1973, **31**, 37.
[177] R. K. McMullan, W. Saenger, J. Fayos, and D. Mootz, *Carbohydrate Res.*, 1973, **31**, 211.
[178] E. A. Lewis and L. D. Hansen, *J.C.S. Perkin II*, 1973, 2081.

guest molecules, the enthalpy and entropy changes were different in a number of cases. Changes in ΔH were largely compensated for by changes in ΔS, due principally to the nature of the solvent. The reaction between gel-forming bacterial β-(1 → 3)-glucans from *Alcaligenes faecalis* and Aniline Blue to form stable colour complexes has been reported.[179] The rate of interaction between two such polysaccharides and the dye in aqueous solutions was shown to be proportional to both the concentration and the gel-forming ability of the polysaccharides. In a study of the ultrastructure of curdlan, a gel-forming glucan from *A. faecalis*, it was shown that the gel is composed of long microfibrils, with apparently a relationship between the length of the microfibrils and the gel strength.[180] Cross-reactions of extracellular polysaccharide of *Lipomyces lipoferus* and *L. starkeyi* in anti-pneumococcal and other antisera have been described.[181] Non-reducing end groups of D-glucuronic acid appear to account for most of the explicable reactions of the polysaccharide of *L. lipoferus*, whereas non-reducing end groups of D-galactose account for many of those of *L. starkeyi*. The *L. starkeyi* polysaccharide is also presumed to have a proportion of its D-glucuronic acid in the form of non-reducing end groups, in view of its strong cross-reaction in anti-pneumococcal type II serum. The glucuronomannans of *L. lipofer* have been purified by way of their Fehling's complexes, ion-exchange chromatography, and gel-permeation chromatography.[182] From the results of chemical and mass-spectral studies, a structure with α-(1 → 3)- and α-(1 → 4)-linkages between the D-mannosyl residues, and with the α-D-glucuronic acid residues linked at O-3 of the D-mannosyl residues, was proposed. Cell suspensions of *Micrococcus sodonensis* have been shown to secrete from seven to ten individual proteins, which include an alkaline phosphatase, a nuclease, and a protease; the appearance of the enzyme activities in the extracellular medium depends on the co-secretion of at least one of several polysaccharides also elaborated by the cells.[183] Evidence was provided to suggest that the polypeptide chains are vulnerable to proteolytic degradation during secretion, but that the co-secretion of polysaccharide protects them from proteolysis. Extracellular polysaccharides synthesized by *Mycobacterium lacticolum* in media containing D-glucose or L-rhamnose were shown to be heteroglycans containing D-glucose and D-glucuronic acid.[184] D-Mannose has been shown *not* to be a constituent of the extracellular polysaccharides elaborated by fast-growing *Rhizobia* cultured on a yeast–mannitol medium;[185] this is in contrast to a previous report which claimed the presence of D-mannose in the polysaccharides.[186] It was suggested that the D-mannosyl residues could have been derived from a contaminating yeast mannan.

A comparative chemical analysis has been presented of the acid exopolysaccharides isolated from seven strains of *Rhizobium* from the taxonomic groups

[179] I. Nakanishi, K. Kimura, S. Kusui, and E. Yamazaki, *Carbohydrate Res.*, 1974, **32**, 47.
[180] A. Koreeda, T. Harada, K. Ogawa, S. Sato, and N. Kasai, *Carbohydrate Res.*, 1974, **33**, 396.
[181] M. Heidelberger and M. E. Slodki, *Carbohydrate Res.*, 1972, **24**, 401.
[182] P. K. Kochetkov, C. E. Gorin, A. F. Sviridov, O. S. Chizov, V. I. Golubev, I. P. Bab'eva, and A. Y. Podel'ko, *Izvest. Akad. Nauk S.S.S.R., Ser. biol.*, 1973, 2304.
[183] J. A. Braatz and E. C. Heath, *J. Biol. Chem.*, 1974, **249**, 2536.
[184] E. V. Gogoleva, N. N. Grechushkina, and N. S. Egorov, *Mikrobiologiya*, 1973, **42**, 409.
[185] B. Humphrey, M. Edgley, and J. M. Vincent, *J. Gen. Microbiol.*, 1974, **81**, 267.
[186] R. W. Bailey, R. M. Greenwood, and A. Craig, *J. Gen. Microbiol.*, 1971, **65**, 315.

Rhizobium meliloti, *Rh. trifolii*, *Rh. phaseoli*, and *Rh. leguminosarum*.[187] Apart from the polysaccharide from *Rh. meliloti*, which contains no uronic acid, no significant differences in carbohydrate composition were reported. Two non-nitrogen-fixing strains, one infective and the other non-infective, elaborated polysaccharides differing from those produced by the infective and nitrogen-fixing strains, as demonstrated by the pattern of periodate oxidation and cation-binding capacity. The carbohydrate composition of the extracellular materials from four strains of *Rh. japonicum* has been reported to be essentially the same for each.[188]

A purified, extracellular, acidic polysaccharide of *Serratia piscatorum* has been shown to exhibit anti-inflammatory activity; it is composed of residues of L-rhamnose, D-galactose, and D-galacturonic acid (2 : 1 : 1), and traces of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, protein, and fatty acid were also found.[189] Ultrasonic treatment degraded the active fraction, which, on further purification, was found to comprise hexose, 2-amino-2-deoxy-D-glucose, and protein.[190] The occurrence of other anti-inflammatory extracellular polysaccharides has been studied.[191] Neutral polysaccharide fractions exhibiting biological activity have been isolated from two *Pseudomonas* sp., a *Bacillus* sp., and an *Agrobacterium* sp. The hydroxy-groups in the macromolecular moiety of *S. piscatorum* exopolysaccharide are considered to play an important role in the manifestation of the anti-inflammatory activity, since the removal of protein and fatty-acid esters did not affect the biological activity.[192] The acidic polysaccharide of *S. piscatorum*, which contains residues of L-rhamnose, D-galactose, and D-galacturonic acid, has been submitted to Smith degradation and methylation analysis.[193] It was suggested from the results that the L-rhamnopyranosyl, D-galactopyranosyl, and D-galactopyranosyluronic acid residues are substituted with glycosidic linkages at O-3, O-3, and O-4, respectively, with the uronic acid residues acetylated at O-2 or O-3, or both. Partial acid hydrolysis of the native polysaccharide gave four acidic oligosaccharides, each of which was isolated and characterized, suggesting the repeating unit (15).

$$\rightarrow 3)\text{-L-Rha}p\text{-}(1 \rightarrow 4)\text{-D-GalUA}p\text{-}(1 \rightarrow 3)\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-D-Gal}p\text{-}(1 \rightarrow \quad (15)$$

Two kinds of L-arabinans possessing anti-carrageenan abscess activity have been isolated from culture filtrates of *Streptomyces fradiae*.[194] Ion-exchange chromatographic procedures separated the polymers on the basis of differences in the contents of D-galacturonic acid. Structural studies indicated that the arabinans are composed of linear chains of α-(1 → 5)-linked L-arabinofuranosyl units, with D-galacturonic acid residues α-linked to the backbone. Periodate

[187] R. Sømme, *Carbohydrate Res.*, 1974, **33**, 89.
[188] B. B. Keele, R. W. Wheat, and G. H. Elkan, *J. Gen. Appl. Microbiol.*, 1974, **20**, 187.
[189] Y. Kita, I. Nakanishi, and M. Isono, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 423.
[190] Y. Kita, S. Igarasi, I. Nakanishi, and M. Isono, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 817.
[191] Y. Kita, M. Isono, A. Misaki, K. Endo, and H. Yamasaki, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 1407.
[192] Y. Kita, I. Nakanishi, M. Isono, K. Endo, and H. Yamasaki, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 1631.
[193] Y. Kita, S. Igarasi, and M. Isono, *Carbohydrate Res.*, 1974, **38**, 239.
[194] H. Arita, H. Tsuzuki, K. Morihara, and J. Kawanami, *J. Biochem.* (*Japan*), 1974, **76**, 861.

oxidation caused a loss of biological activity. A glucan and a fructan, both of molecular weight 2×10^7, have been isolated from *Streptococcus mutans*.[195] Structural studies on the glucan showed it to be of the general type elaborated by *Streptococcus* sp., since it is composed of α-D-glucosyl residues in 3-, 6-, and 3,6-linkages. However, the fructan did not appear to be of the levan-type, with (2 → 6)-linkages, but of the inulin-type, with (2 → 1)-linked β-D-fructofuranosyl residues, some of which are branched through O-6.

Miscellaneous Bacterial Polysaccharides.—The identification of labelled ^{14}C-products from the utilization of D-[U-^{14}C]glucose by washed beech litter has shown that they are mostly microbial polysaccharides, such as arabinans and glucans.[196] Determination of the amount of microbial polysaccharides, which act in the turnover of free D-glucose present in the litter, could be used as a measure of the biomass of bacteria and fungi. A crude enzyme system isolated from *Acetobacter xylinum* incorporated radioactivity from UDP-D-[^{14}C]glucose into an alkali-insoluble polysaccharide (presumed to be cellulose), an alkali-soluble polysaccharide, and three lipid intermediates, namely a lipid diphosphate D-glucose, a lipid diphosphate cellobiose, and a lipid monophosphate D-galactose.[197] The biosynthesis of the slime produced by *Bacterium aceris* has been shown to require the presence in the growth medium of sucrose and raffinose, which function as donors of monosaccharide residues, as carbon compounds necessary for anabolism, and as energy sources for protein biosynthesis.[198] Protoplast membranes isolated from the cells of *Bacillis cereus* harvested late in their exponential-growth phase, just prior to sporogenesis, contained 6% carbohydrate as neutral hexoses.[199] A slime substance, which was characterized as a levan, has been isolated from fish-jelly products contaminated by *B. licheniformis*.[200] No differences could be detected in the amino-acid compositions of cell-wall fractions of two strains of *Clostridium perfringens*, and, although both cell walls contain D-glucosyl, D-galactosyl, and 2-amino-2-deoxy-D-glucosyl residues, L-rhamnose was detected as a component of only one of them.[201] It was suggested that the carbohydrate composition of the cell wall could serve as one of the factors determining the serological specificities of the strains. Strains of *C. perfringens* from different serological groups were found to contain a common antigen, which is polysaccharide in nature, as well as strain-specific antigens.[202] It was suggested that the cell-wall polysaccharide of *C. perfringens* type A could determine the antigenic properties of this organism. Evidence has been presented that L-iduronic acid is a constitutent of the type-specific polysaccharide of *C. perfringens* Hobbs 10.[203] A toxin isolated from apple tissues infected by *Erwinia amylovora* was identified as a galactan of molecular weight 1.7×10^5.[204]

[195] K. G. Rosell and D. Birkhed, *Acta Chem. Scand.* (*B*), 1974, **28**, 589.
[196] J. Mayaudon, L. Guillemot, and C. Bellinck, *Ann. Microbiol.*, 1974, **125A**, 201.
[197] R. C. Garcia, E. Recondo, and M. Dankert, *European J. Biochem.*, 1974, **43**, 93.
[198] E. T. Tuleuova, *Mikrobiologiya*, 1974, **43**, 363.
[199] T. C. Beaman, H. S. Pankratz, and P. Gerhardt, *J. Bacteriol.*, 1974, **117**, 1335.
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[201] G. M. Shaposhnikova, G. K. Shipitsyna, M. V. Ispolatovskaya, V. A. Blogoveschensky, and E. P. Zemiyanitskaya, *Zhur. Mikrobiol. Epidemiol. Immunobiol.*, 1973, **50**, 36.
[202] M. V. Ispolatovskaya, G. M. Shaposhnikova, and E. P. Zemiyanitskaya, *Zhur. Mikrobiol. Epidemiol. Immunobiol.*, 1973, **50**, 40.
[203] L. Lee and R. Cherniak, *Carbohydrate Res.*, 1974, **33**, 387.
[204] N. R. Goodman, J. S. Huang, and P. Y. Huang, *Science*, 1974, **183**, 1081.

The polysaccharide toxin was produced neither *in vitro* nor *in vivo* by avirulent strains of the pathogen, and was considered to be produced from the interaction of a susceptible host with a virulent pathogen. The polysaccharide antigen produced by *Eubacterium saburreum* has been shown to be a linear molecule composed of β-(1 → 6)-linked D-*glycero*-D-*galacto*-heptopyranose residues, 65% of which carry an *O*-acetyl group at the 7-position.[205] A membrane-associated mannan of *Micrococcus lysodeikticus* has been found to have properties in common with lipoteichoic acids, forming micelles in aqueous solution and possessing a negative charge that makes it capable of binding cations.[206] The mannan is acylated, and is presumably attached to the cytoplasmic membrane by intercalation of its fatty acid residues in a manner similar to that suggested for teichoic acids. Concanavalin A was found to react with three antigenic polysaccharides present in culture filtrates of *Mycobacterium tuberculosis*.[207] Using direct precipitation and affinity chromatography, the reactive polysaccharides were separated from the non-reactive ones; two of the antigens were tentatively classified as an arabinogalactan and an arabinomannan, respectively. Extraction of the purified walls of *M. tuberculosis* with alkali released a polymer composed of L-arabinose, D-galactose, and D-glucose and a material composed of equal proportions of L-arabinose and phosphate.[208] The cell walls of five species of *Mycobacterium* have been shown to contain D-arabino-D-galactans having similar chemical and immunological properties.[209] The polysaccharides are considered to have ramified structures, with eleven to sixteen sugar residues in repeating units consisting of (1 → 5)- and (1 → 2)-α-D-arabinofuranosyl residues and of either (1 → 4)-β-D-galactopyranosyl or (1 → 5)-β-D-galactofuranosyl residues. Side-chains are terminated with D-arabinofuranosyl residues and are attached to the main chain at O-3 of the D-arabinosyl residues and, probably, at O-6 of the D-galactosyl residues. The D-arabinofuranosyl side-chains are responsible for serological activity. These features are accommodated in the general structure (16). The arabinogalactan reacted not only with antisera against mycobacterial cell walls but also with those against *Corynebacterium diphtheriae* and *Nocardia asteroides*, suggesting that the polymer is a common antigen of these bacterial groups.

Polysaccharide antigens isolated from Meningococcus groups A and C have been shown to contain protein and to have a molecular weight of 1.5×10^5.[210] The antigen present in cerebrospinal fluids and sera of patients with group A meningococcal meningitis has been shown, by counter-current immunoelectrophoresis, to be a polysaccharide of high molecular weight.[211] Its composition was deduced from its immunochemical identity with the known group A meningococcal polysaccharide, which had previously been shown to be a polymer of *N*- and *O*-acetylated residues of 2-amino-2-deoxy-D-mannose phosphate. A

[205] J. Hoffman, B. Lindberg, S. Svensson, and T. Hofstad, *Carbohydrate Res.*, 1974, **35**, 49.
[206] D. A. Powell, M. Duckworth, and J. Baddiley, *F.E.B.S. Letters*, 1974, **41**, 259.
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[209] A. Misaki, N. Seto, and I. Azuma, *J. Biochem.* (*Japan*), 1974, **76**, 15.
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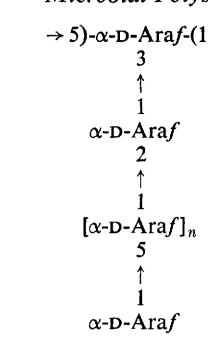
[211] B. M. Greenwood and H. C. Whittle, *Clin. Exp. Immunol.*, 1974, **16**, 417.



(16)

mucoid-type strain of *Pseudomonas aeruginosa* produced an alginate-like substance consisting of mannuronic acid and glucuronic acid and small amounts of protein and nucleic acid.[212] Although γ-irradiation of *Salmonella typhi* caused a decrease in the toxicity and the immunological properties, the antigenic and immunogenic properties differed little from those of non-irradiated antigens.[213] The quantitative compositions of the irradiated and non-irradiated specific polysaccharides were identical, suggesting a comparatively high stability for the polysaccharide component of the endotoxins. Purification of a protective *Staphylococcus* antigen has been achieved by trypsinization of the cells and the removal of non-antigenic, nucleic-acid materials by gel-permeation chromatography, which revealed two fractions having immunogenic activity, one consisting mainly of polysaccharide and the other of a protein–polysaccharide–nucleic acid complex.[214] Immunochemical studies of type IV and two group-like (Z_4 and Z_5) carbohydrate antigens of minute streptococci have shown that D-galactose is a determinant group.[215] Analyses and inhibition reactions on oligosaccharides obtained by hydrolysis of the type IV antigen indicated that the trisaccharides β-D-galactosyl-D-glucosyl-D-galactose and β-D-galactosyl-D-glucosyl-L-rhamnose are determinant groups. Two antigens found in cell-wall extracts of nephritogenic group A *Streptococcus* before an increase in virulence have been identified as a group polysaccharide and a teichoic acid, respectively.[216] By use of a radiolabelled, antigenically univalent hapten derived from a streptococcal group A carbohydrate, the affinity of serum antibodies to the group A carbohydrate was studied by a precipitation technique.[217]

A *S. mutans* group *d* cell-wall polysaccharide containing D-galactose and D-glucose (2 : 1) has been purified by ion-exchange and gel-permeation chromatography.[218] The antigen was shown to contain two serologically active sites on the same molecule, one specific for group *d* and the other common to both groups *d* and *a*. The immunological specificity for the polysaccharide site of group *d*

[212] T. Murakawa, *Jap. J. Microbiol.*, 1973, **17**, 513.
[213] I. V. Shibayaeva, A. V. Izvekova, K. K. Ivanov, and M. A. Tumanyan, *J. Hyg. Epidemiol. Microbiol. Immunol.*, 1973, **17**, 357.
[214] K. R. Tolovskaya and A. K. Akatov, *Zhur. Mikrobiol. Epidemiol. Immunobiol.*, 1974, **51**, 83.
[215] J. M. N. Willers, M. F. Michel, and R. Benner, *J. Microbiol. Serol.*, 1973, **39**, 609.
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[217] S. T. Shulman and E. M. Ayoub, *J. Clin. Invest.*, 1974, **54**, 990.
[218] R. Linzer and H. D. Slade, *Infection and Immunity*, 1974, **10**, 361.

depends on terminal D-galactosyl units. Comparative evaluation of two polysaccharide preparations isolated from *Vibrios El Tor* showed that they are both heteropolymers containing D-galactose, D-glucose, D-mannose, 2-amino-2-deoxy-D-glucose, and D-ribose.[219] A temperature-dependent alteration in the synthesis of the cell-wall polysaccharides by certain streptococcal variants has been demonstrated.[220] Immunochemical data were presented detailing the changes associated with this temperature-related alteration, which appears to be a property of strains with intermediate characteristics.

Fungal Polysaccharides

The biological properties and functions, biosynthesis, and morphology of fungal cell-wall glycoproteins and peptidopolysaccharides have been reviewed.[221] A review on the chemistry and biochemistry of fungal cell walls includes sections on chitin, chitosan, carbohydrate–protein polymers, complex polysaccharides, and glucans.[222]

Glucans.—When *Aspergillus niger* cells were incubated with D-[^{3}H]glucose under conditions favourable to the accumulation of nigeran, autoradiographic techniques revealed that essentially all the label was localized around the hyphal perimeter.[223] Although α-(1 → 3)-D-glucan and α-1,3-glucanase have been shown to be indispensable for fructification in *A. nidulans*, 2-deoxy-D-*arabino*-hexose influenced the synthesis of α-(1 → 3)-glucan by inhibiting the α-1,3-glucanase, either as a secondary effect or as a consequence of disturbing the metabolism of the cells.[224] The formation of cleistothecia and the production of α-(1 → 3)-glucan have been shown to be inversely related to conidiation. From a study of a number of morphogenetic mutants of *A. nidulans*, it was demonstrated that the small quantity of α-(1 → 3)-glucan present correlated with the low α-(1 → 3)-glucanase activity and the absence of cleistothecia.[225]

The macromolecular structure and morphology of native glycogen particles isolated from *Candida albicans* were shown to be similar to those of the corresponding polymer from rat liver.[226] A water-soluble, cell-wall glucan from *Cladosporium herbarum* has been studied by periodate oxidation, Smith degradation, and methylation techniques, which showed it to be a linear molecule comprising (1 → 3)- and (1 → 4)-D-glucopyranosyl units.[227] Coriolan, a glucan produced by *Coriolus versicolor* that possesses antitumour activity, has a highly branched structure comprising a main backbone of (1 → 3)-linkages and either (1 → 6)-linked or (1 → 3)- and (1 → 6)-linked side-chains.[228] Biosynthetic studies on the β-glucan of *Cochliobolus miyabeanus* have demonstrated the incorporation

[219] L. N. Savkina, E. P. Efimtseva, and Z. V. Ermolieva, *Antibiotiki*, 1974, **19**, 535.
[220] E. M. Ayoub and B. A. Dudding, *J. Exp. Med.*, 1973, **138**, 117.
[221] J. E. Gander, *Ann. Rev. Microbiol.*, 1974, **28**, 103.
[222] R. J. Sturgeon in 'Plant Carbohydrate Biochemistry', ed. J. B. Pridham, Phytochemical Society Symposia, Academic Press, London and New York, 1974, No. 10, p. 219.
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[224] B. J. M. Zonneveld, *Dev. Biol.*, 1973, **34**, 1.
[225] B. J. M. Zonneveld, *J. Gen. Microbiol.*, 1974, **81**, 445.
[226] H. Yamaguchi, Y. Kanda, and K. Iwata, *J. Bacteriol.*, 1974, **120**, 441.
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[228] T. Miyazaki, T. Yadomae, M. Sugiura, H. Ito, K. Fujii, S. Naruse, and M. Kunihisa, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1739.

of D-glucose from UDP-D-[^{14}C]glucose into non-reducing, terminal positions *via* β-(1 → 3)-linkages and into internal residues *via* β-(1 → 6)-linkages.[229] The metabolism of glycogen during differentiation in *Dictyostelium discoideum* has been reviewed.[230] A water-soluble glucan isolated from the sclerotium of *Grifora umbellata* was shown by a combination of periodate oxidation, Smith degradation, methylation, and i.r. techniques to be branched at O-6 or O-3 of the D-glucosyl residues and to possess β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-linkages.[231] The cell-wall starch of *Hericium abietis* is apparently similar to the 'capsular' starch-like polysaccharide excreted by yeasts of the genus *Cryptococcus*.[232] Pullulan has been synthesized from sucrose by acetone-dried cells, or from UDP-D-glucose by cell-free preparations, of *Pullularia pullulans*.[233] A lipid-containing D-glucose residue was formed during the reaction, and its participation as an intermediate in the biosynthesis was assumed. Periodate oxidation and enzymic degradation have shown that the insoluble glucan preparations from *Kloeckera apiculata*, *Schizosaccharomyces pombe*, *Saccharomyces fragilis*, and *Saccharomyces fermentati* contain branched β-(1 → 3)- and β-(1 → 6)- structures.[234] The regulation of glycogen synthesis in yeasts by allosteric effectors has been described.[235] A method has been reported for the isolation of anucleated yeast protoplasts, which, although seeming to contain all other basic structures in the cytoplasm, were unable to synthesize glucan.[236] This contrasts with ordinary nucleated protoplasts, which can synthesize glucan fibrils even after the inhibition of protein synthesis by cycloheximide. A decrease in the production of the extracellular glucan of *Sclerotium rolfsii* has been demonstrated on the addition of lactose to a liquid glucose-synthetic medium.[237] Scleroglucan, an antitumour polysaccharide from *S. glucanium* that is effective against sarcoma 180 in mice, has been shown to be composed of a main chain of β-(1 → 3)-linked D-glucopyranosyl units, with every third or fourth unit carrying a β-(1 → 6)-linked D-glucopyranosyl unit.[238] Removal of the branches, by a combination of periodate oxidation and Smith degradation, produced an inactive glucan, implying that linearity alone does not account for the antitumour activity. The co-operative action of *endo-* and *exo*-β-(1 → 3)-glucanases from two parasitic fungi has been used to obtain a complete breakdown of the cell-wall glucan of *S. sclerotiorum*.[239] When acting alone, the *endo*-β-(1 → 3)-glucanase had a restricted action on the glucan, yielding, *inter alia*, a trisaccharide tentatively identified as 6^2-β-glucosyllaminaribiose.

Mannans.—Aspects of the structure, biosynthesis, and genetic control of yeast mannans have been reviewed.[240] Chemical investigations have indicated that the

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[230] B. E. Wright, P. Rosness, T. H. D. Jones, and R. Marshall, *Ann. New York Acad. Sci.*, 1973, **210**, 51.
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[234] D. J. Manners, A. J. Masson, and J. C. Patterson, *J. Gen. Microbiol.*, 1974, **80**, 411.
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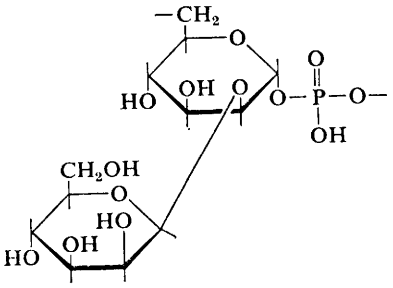
[236] M. Kopecka, M. Gabriel, and O. Nečas, *J. Gen. Microbiol.*, 1974, **81**, 111.
[237] Y. Okon, I. Chet, N. Kislev, and Y. Henis, *J. Gen. Microbiol.*, 1974, **81**, 145.
[238] P. P. Singh, R. L. Whistler, R. Tokuzen, and W. Nakahara, *Carbohydrate Res.*, 1974, **37**, 245.
[239] D. Jones, A. H. Gordon, and J. S. D. Bacon, *Biochem. J.*, 1974, **140**, 47.
[240] C. E. Ballou, *Adv. Enzymol.*, 1974, **40**, 239.

mannan from *Alternalia kikuchiana* has a branched structure in which an α-(1 → 6)-linked D-mannan backbone is branched by α-(1 → 2)- and α-(1 → 3)-linkages.[241] This mannan was also shown to be serologically cross-reactive with the mannans of *Saccharomyces* and *Candida*. Microsomal preparations from *Asperigillus niger* have mediated the transfer of D-mannosyl units from GDP-D-mannose to endogenous lipids, whose chromatographic and chemical properties were similar to those of polyprenol phosphate.[242] The ^{13}C n.m.r. spectrum of *Hansenula capsulata* mannan in D_2O was expanded on the addition of lanthanide ions.[243] With the aid of a parallel study on the structurally related α-D-mannose 1- and 6-phosphates, the signal displacements were interpreted in terms of a structure (17) based on a repeating unit of 2-*O*-β-D-mannopyranosyl-α-D-



(17)

mannopyranose joined by (1 → 6)-phosphoric diester linkages. Both the cell-wall and the extracellular phosphomannans of *H. holstii* are composed of mixtures of several polysaccharides of molecular weight 1×10^6, which differ in the degrees of phosphorylation and the structural arrangements.[244] The cell-wall mannan comprises two branched polysaccharides containing α-(1 → 2)- and α-(1 → 3)-linkages; a third polysaccharide is phosphorylated and contains β-linkages as well as α-linkages. At least three extracellular polysaccharides exist, all comprising α-(1 → 6)-linked backbones to which α-(1 → 2)- and α-(1 → 3)-side-chains of various lengths are attached. When the *H. holstii* was grown under phosphorus-limiting conditions, both the isolated cell wall and the extracellular mannans had a lower phosphate content, with *O*-acetyl groups replacing most of the phosphate.[245] Both phosphomannans had a branched structure similar to that of the wall mannan of *Saccharomyces cerevisiae*, the main difference residing in the incorporation of *O*-acetyl groups. Radioactive products derived from the transfer of D-[^{14}C]mannosyl residues from GDP-D-[^{14}C]mannose to endogenous acceptors by a *H. holstii* particulate enzyme preparation have been solubilized by proteolytic digestion, yielding a mixture of glycopeptides containing D-[^{14}C]-

[241] I. Azuma, S. Negoro, F. Kanetsuna, Y. Yamamura, H. Miyaji, and A. Misaki, *Jap. J. Microbiol.*, 1971, **15**, 373.
[242] R. C. P. Létoublon, J. Comte, and R. Got, *European J. Biochem.*, 1973, **40**, 95.
[243] P. A. J. Gorin and M. Mazurek, *Canad. J. Chem.*, 1974, **52**, 3070.
[244] G. San Blas and W. L. Cunningham, *Biochim. Biophys. Acta*, 1974, **354**, 233.
[245] G. San Blas and W. L. Cunningham, *Biochim. Biophys. Acta*, 1974, **354**, 247.
[246] R. K. Bretthauer and G. C. Tsay, *Arch. Biochem. Biophys.*, 1974, **164**, 118.

mannose.[246] Elimination and reduction experiments indicated that D-mannose-containing oligosaccharides are linked *O*-glycosidically to serine or threonine residues.

A mannan from *H. polymorpha* having an α-D-glucosyl phosphate residue as an important immunochemical determinant has been shown to possess a modified block-type structure in which α-(1 → 6)- and α-(1 → 2)-D-mannosyl residues form part of the backbone.[247] Methylation analyses showed that most of the (1 → 6)-linked D-mannosyl units are otherwise unsubstituted, and that the branch points in the mannan involve positions 2 and 6, with an average chain-length of eight units. In contrast to the (1 → 6)-linked backbone in *S. cerevisiae* mannan, that of *H. wingei* mannan appears to comprise (1 → 2)-, (1 → 3)-, and (1 → 6)-linkages.[248] Most of the mannose is present in the form of large polysaccharide units, probably attached to asparagine residues in the protein. Immunochemical studies on the mannan revealed that the homologous precipitation reaction is due in part to the terminal trisaccharide α-D-Man-(1 → 3)-α-D-Man-(1 → 3)-D-Man, whereas the remainder involves an acid-labile structure in the mannan. The sexual agglutination factor on *H. wingei* cells has been purified following its release from the cell surface by proteolysis.[249] The preparation (mol. wt. 9.6×10^5) was composed of 85% carbohydrate (mostly D-mannose), protein, and phosphate in a structure having 90% of the carbohydrate linked to the protein in the form of manno-oligosaccharides containing up to fifteen units. A specific, antigenic mannan has been found in several *Kluyveromyces* spp., and in *Candida pseudotropicalis* and *Saccharomyces chevalieri*.[250] The mannans showed a high cross-reactivity with *S. cerevisiae* antiserum, presumably ascribable to the presence of a determinant group common to the *Kluyveromyces* and *Saccharomyces* species. Mannan has been cross-linked with epichlorohydrin to produce an immunoadsorbent for the corresponding yeast antibodies.[251] Immunochemical studies on mannans of the genus *Saccharomyces* have shown that mannotetraose is the immunodominant group in eight of the strains investigated, although different structural features and immunochemical properties were detected in *S. rouxii* and *S. rosei*.[252] The products of acetolysis of the mannans of *S. chevalieri*, *S. italicus*, *S. diastaticus*, and *S. carlsbergensis* were distinguished from those of *S. cerevisiae* by the presence of a pentasaccharide in addition to the mono-, di-, tri-, and tetra-saccharides.[253] Digestion of two of the mannans with mannanase yielded residues comprising linear (1 → 6)-linked polysaccharides, thus establishing the structural relationship between these mannans and that from *S. cerevisiae*. The principal immunochemical determinant on the cell surface in all strains appears to be a terminal (1 → 3)-linked α-D-mannosyl unit. The mannan from *S. cerevisiae* has been shown to inhibit sarcoma 180 in mice; after successive injections of D-[^{3}H]mannan, the radioactivity was shown to accumulate predominantly into the reticuloendothelial

[247] P. N. Lipke, W. C. Raschke, and C. E. Ballou, *Carbohydrate Res.*, 1974, **37**, 23.
[248] P. H. Yen and C. E. Ballou, *Biochemistry*, 1974, **13**, 2420.
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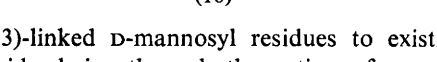
[250] J. Šandula, A. Kocková-Kratochvílová, and D. Šikl, *J. Gen. Microbiol.*, 1974, **83**, 339.
[251] J. Šandula and Ľ. Kuniak, *J. Chromatog.*, 1974, **91**, 293.
[252] J. Šandula and A. Vojtková-Lepšíková, *Folia Microbiol.*, 1974, **19**, 94.
[253] C. E. Ballou, P. N. Lipke, and W. C. Raschke, *J. Bacteriol.*, 1974, **117**, 461.

tissues.[254] The incorporation of mannan labelled with D-[1-^{3}H]mannose into the growing cell walls of *S. cerevisiae* has been followed by means of high-resolution autoradiography; in the first stage of budding, new mannan is incorporated uniformly over the whole surface of the emerging bud, which at this stage is nearly spherical.[255] The ellipsoidal shape of the cell walls is considered to result from a combination of polarized tip growth and non-polarized, spherical extension. Pulse-labelling techniques have demonstrated that D-[^{3}H]mannose, which is incorporated exclusively as mannan, is fixed primarily in the cytoplasmic space, wherefrom it is transported to the cell wall.[256] Experiments with separated membrane fractions from *S. cerevisiae* strongly support the hypotheses that the plasmalemma is not directly involved in the biosynthesis of mannan and that the polymerization of D-mannosyl residues takes place at the membranes of the endoplasmic reticulum. No gross differences could be detected in the structures of the mannans isolated from bud scars and cell walls of *S. cerevisiae*, although the molecular weights of mannans isolated by enzymic methods were greater than those of mannans isolated by chemical methods.[257] Analysis of *S. cerevisiae* X2180 phosphomannan has shown that it contains phosphorylated side-chains possessing the structure (18).[258] This unit apparently arises in the mannan by the



(18)

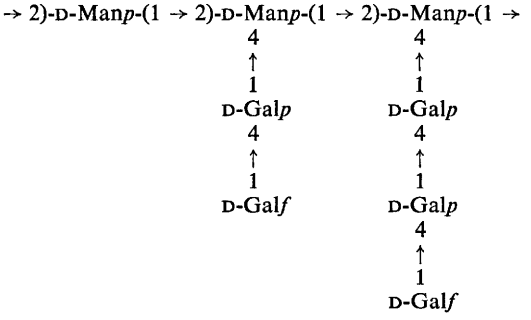
addition of α-(1 → 3)-linked D-mannosyl residues to existing mannosylphosphorylmannotriose side-chains through the action of an α-(1 → 3)-mannosyltransferase. It is considered that immunochemical differences between this strain and a closely related strain (4484-24D) are determined by the presence or absence of this single enzyme. In its absence, the D-mannosylphosphate groups, which are added to the mannotriose side-chains through the action of a mannosylphosphatetransferase, act as characteristic antigenic determinants of the mannan, as in the latter strain.

Yeast vacuole preparations from *S. cerevisiae* have been found to be greatly enriched in chitin-synthetase activating factor and essentially devoid of chitin-synthetase zymogen.[259] The latter was found in another membrane-rich fraction, thus supporting earlier claims that the activating factor and the zymogen reside in different organelles. The protein inhibitor of yeast protease that activates chitin-synthetase zymogen has been purified and found to have a molecular weight of 8.5×10^3.[260]

Cell-wall glycopeptides from two *Candida albicans* serotypes have been separated by gel-permeation chromatography into a mannan-peptide and a glucan-peptide.[261] β-Elimination studies with alkali showed that the disaccharide

[254] H. Hojo, M. Uchiyama, and S. Suzuki, *Yakugaku Zasshi*, 1973, **93**, 947.
[255] V. Farkaš, J. Kovařík, A. Košinová, and Š. Bauer, *J. Bacteriol.*, 1974, **117**, 265.
[256] A. Košinová, V. Farkaš, S. Machala, and Š. Bauer, *Arch. Mikrobiol.*, 1974, **99**, 255.
[257] D. A. Bush and M. Horisberger, *J. Biol. Chem.*, 1973, **248**, 1318.
[258] L. Rosenfeld and C. E. Ballou, *J. Biol. Chem.*, 1974, **249**, 2319.
[259] E. Cabib, R. E. Ulane, and B. Bowers, *J. Biol. Chem.*, 1973, **248**, 1451.
[260] R. E. Ulane and E. Cabib, *J. Biol. Chem.*, 1974, **249**, 3418.
[261] N. Kolarova, L. Masler, and D. Šikl, *Biochim. Biophys. Acta*, 1973, **328**, 221.

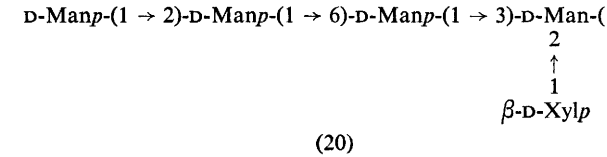
units are linked *O*-glycosidically to threonine residues. The major water-soluble polysaccharide of *Cladosporium herbarum* comprises D-galactose and D-mannose (2 : 3); periodate oxidation, Smith degradation, and methylation analysis showed it to have a structure (19), containing a backbone of (1 → 2)-linked D-mannosyl



(19)

residues having (1 → 4)-linked D-galacto-pyranosyl and -furanosyl units variously joined to O-4.[262]

A qualitative study based on cytochemical and *X*-ray data has been reported on the distribution of cellulose in the cell wall of members of the genera *Ceratocystis* and *Euphorium*.[263] An enzyme system isolated from *Cryptococcus laurentii* catalysed the transfer of glycosyl units from sugar nucleotides to exogenous acceptors, resulting in the stepwise synthesis of the heteroglycan (20).[264] The



(20)

same enzyme preparation also transferred D-[^{14}C]mannose from GDP-D-[^{14}C]-mannose to an endogenous glycoprotein acceptor, which led to the suggestion that the pentasaccharide is synthesized *de novo* as an integral part of the cell-envelope glycoprotein. The presence of β-D-glucans, chitin, and protein has been reported in the cell walls of *Dendryphiella vinosa*; small proportions of D-galactose and D-mannose are also present and are linked to D-glucose in heteroglycans.[265] Two different extracellular glycans are produced in the growth phase of the cellular slime-mould *Dictyostelium discoideum*.[266] One polysaccharide contains D-glucose, D-glucuronic acid, D-mannose, and D-galactose (10 : 4 : 1 : 1), which, together with D-galacturonic acid and 2-amino-2-deoxy-D-glucose, are also components of the other polysaccharide. A report has appeared on the host-mediated antitumour effect of the (1 → 3)-β-D-glucan from *Flammulina velutipes*

[262] T. Miyazaki and Y. Naoi, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1360.
[263] T. R. Jewell, *Mycologia*, 1974, **66**, 139.
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[265] R. Bonaly, *Carbohydrate Res.*, 1972, **24**, 355.
[266] H. Yamada, T. Yadomae, and T. Miyazaki, *Biochim. Biophys. Acta*, 1974, **343**, 371.

and on its relation to biogenetic amines in tissues under vitamin-B_6 deficiency.[267] The cell walls of *Morchella* sp. have been shown to contain 17% of non-nitrogenous polysaccharides, comprising D-glucose, D-galactose, and D-mannose, and protein and chitin.[268]

Comparative studies of the cell-wall compositions of yeast-like and mycelial forms of *Paecilomyces viridis* have shown that, in addition to D-glucose, D-galactose, and D-mannose, the former also contains equal proportions of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose, whereas the mycelial form contains only 2-amino-2-deoxy-D-glucose.[269] Differences in the patterns of sugars released on alkaline treatment of the two forms were also noted. An extracellular peptidophosphogalactomannan from *Penicillium charlesii* has previously been shown to comprise a polysaccharide of high molecular weight and several oligosaccharides attached to the peptide through serine and threonine residues.[270] Antibodies to guinea-pig sera were directed against the polysaccharide, but not against the peptide or oligosaccharides. It appeared that D-mannopyranosyl residues associated with unidentified acid-labile material are the principal immunodeterminant groups which bind to guinea-pig sera.

A polysaccharide composed of D-galactose and malonic acid (3 : 1) has been isolated from *P. citrinum*.[271] Evidence from ^{1}H n.m.r. spectroscopy, optical and i.r. data, and classical structural studies suggested a repeating (1 → 5)-β-D-galactofuranose structure esterified with malonic acid at either O-2 or O-3. The only significant difference in the cell-wall compositions of the chytrids *Phlyctochytrium arcticum* and *Rhizophydium patellarium* appears to be in the ratio of chitin to other carbohydrates present.[272] The main differences between the yeast-like and mycelial forms of *Histoplasma capsulatum* appear to be in the amount and the nature of the glucan components of the cell walls, the yeast form containing a large amount of an alkali-soluble glucan with α-(1 → 3)-linkages.[273] Chitin microfibrils are located mainly in the inner portion of the cell walls of both forms, whereas α-glucan fibres are found only in the outer portion of the cell wall of the yeast form. Serologically active galactomannans have been isolated from whole cells of *H. capsulatum*, *H. duboisii*, *Paracoccidiodes brasiliensis*, and *Blastomyces dermatidis*.[274] Their structural features were examined by methylation, which showed that each of the galactomannans contains a main chain of (1 → 6)-linked D-mannopyranosyl residues with D-galactofuranosyl and D-mannopyranosyl residues as non-reducing termini in the side-chains. Highly viscous polysaccharides are produced by *Rhodotorula glutinis*, and, after removal of a contaminating mannan, the major component was identified as a fucogalactan

[267] M. Sano, I. Tomita, T. Ozawa, T. Ikekawa, M. Tanaki, and F. Fukuoka, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2090.
[268] J. Ruíz-Herrera and E. Osorio, *J. Microbiol. Serol.*, 1974, **40**, 57.
[269] Z. Barath and V. Betina, *Biologia*, 1972, **27**, 485.
[270] J. E. Gander and J. A. Rudbach, *Immunochemistry*, 1973, **10**, 81.
[271] T. Kohama, M. Fujimoto, A. Kuninaka, and H. Yoshino, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 127.
[272] S. Gerhart, D. J. S. Barr, and H. K. Morita, *Mycologia*, 1974, **66**, 107.
[273] F. Kanetsuna, L. M. Carbonell, F. Gil, and I. Azuma, *Mycopath. Mycologia Applicata*, 1974, **54**, 1.
[274] I. Azuma, F. Kanetsuna, Y. Tanaka, Y. Yamamura, and L. M. Carbonell, *Mycopath. Mycologia Applicata*, 1974, **54**, 111.

containing equimolar proportions of L-fucose and D-galactose.[275] By decreasing the temperature of cultivation of *R. glutinis*, it was shown that the synthesis of lipids is impaired but that of polysaccharides is stimulated.[276] Polysaccharides of the spores of *Saccharomyces cerevisiae* contain all the sugar components present in polysaccharides of the vegetative cells, but in different proportions.[277] The wall structure of *Schizosaccharomyces pombe* has been found to differ in several respects from that of budding ascomycetous yeasts (*e.g. S. cerevisiae*).[278] The presence of α-glucan is considered to denote a close relationship with the mycelial Ascomycetes, and, in the absence of mannan, the galactomannan present in *Schiz. pombe* is indicated to be involved in fission processes. A combination of β-glucanase and chitinase had a synergistic effect in degrading the cross-wall of *Schiz. commune*, where chitin is probably embedded in an alkali-insoluble glucan.[279] A homogeneous, acidic heteroglycan containing residues of D-xylose, D-glucuronic acid, D-mannose, and acetic acid has been isolated from *Tremella fusiformis*.[280]

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5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

BY R. D. MARSHALL

Introduction

The ability to isolate and study the properties of cell membranes by physical methods,[1] and the means of fractionating them,[2] has led to consideration of the importance of glycoproteins, glycolipids, and glycosaminoglycans in many of the properties of the cell surface, including contact inhibition.[3] The functions of the membrane glycoproteins have been discussed as part of a more general symposium.[4] It has also been suggested that cell-surface carbohydrates are involved in cell recognition, interaction, and adhesion.[5] However, no detectable amount of galactosyltransferase could be detected on the cell surface of cultured, baby-hamster kidney cells,[6] as would be required by the hypothesis that these enzymes have a general role in cell adhesion and contact inhibition. The finding of an antigen closely similar to the Tamm–Horsfall glycoprotein as a product of baby-hamster kidney cells (BHK 21/C13) suggests that the latter may be derived from epithelioid cells.[7] The need for care in interpreting the results of experiments in which sugars are incorporated into the surface of cultured cells has been emphasized by the finding of non-specific adsorption to the cells of L-fucose and 2-amino-2-deoxy-D-glucose.[8] The blood-group and Forsmann antigens[9] and the binding sites for lectins[10] lie on the cell surface, as discussed by a number of workers.[11] Much of the interest in the biochemistry of cancer has centred on the way in which the cell surface, particularly the macromolecular components thereof containing sugars, is involved.[12, 13] The use of tumour-associated,

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[3] R. D. Noonan and M. M. Burger, *Progr. Surface Membrane Sci.*, 1974, **8**, 245.
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[5] S. Roth, *Quart. Rev. Biol.*, 1973, **48**, 541.
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[12] Editorial in *Science*, 1974, **183**, 1279.
[13] V. N. Nigam and A. Cantero, *Adv. Cancer Res.*, 1973, **17**, 1.

glycoprotein antigens, which are membrane components, as diagnostic markers is still being explored.[14]

The role of calcium ions, mediated through glycoproteins, in various recognition phenomena has been emphasized; they play a part in the action exerted by insulin,[15] and there is evidence that the mitogenic activity of lectins and of the Streptomyces antibiotic A23187, a monobasic carboxylic acid of molecular weight 512,[16] results from the ability of these substances to increase the permeability of the lymphocyte membrane to calcium ions.[17]

The way in which lipid intermediates may be involved in the biosynthesis of glycoproteins in animal cells is under investigation. An enzyme present in the rough endoplasmic reticulum of liver appeared to catalyse *in vitro* the transfer of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose to an appropriate asparagine sequon.[18, 19] The possibility that a lipid intermediate may be needed *in vivo*, but not *in vitro*, has been discussed.[18] The synthesis of dolichol-linked oligosaccharides catalysed either by pig-liver microsomes [20] or by regenerating rat liver [21] has been discussed in light of the possibility that the products might be precursors of the carbohydrate moieties of glycoproteins. Polyprenylmannosyl phosphates isolated from calf pancreas and from human lymphocytes have been shown to contain a β-linked D-mannosyl residue.[22] A polyisoprenylxylosyl phosphate can be formed *in vitro* from UDP-D-xylose under the catalytic influence of hen oviduct membrane.[23]

Radioimmunoassay procedures for a number of glycoproteins, including hormones and tumour-associated antigens, have become available.[24] Automated procedures for the assay of proteins, hexoses, uronic acids,[25] and siliac acid [26, 27] in column effluents will facilitate studies on glycoproteins, as will the assay of hexosamines by g.l.c. of the deaminated products.[28]

The use of 1,10-phenanthroline as a reagent for sialic acid is not recommended.[29–31]

The production of 3-formylpyruvic acid from sialic acid in the Warren reaction probably requires direct interaction of the primary product (1) with thiobarbituric acid in order to effect cleavage. The primary product does not appear to undergo

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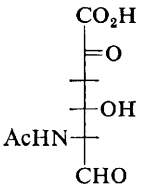

(1)

a retro-aldol fission during the reaction; indeed, 4-substituted sialic acids gave a red colour in the Warren reaction.[32, 33]

An improved method has been reported for the synthesis of ^{14}C- or ^{3}H-labelled *N*-acetylneuraminic acids, which are needed in many studies. The methyl β-glycoside of neuraminic acid, isolated from the methanolysis products of edible bird-nest substance, has been acetylated with radiolabelled acetic anhydride.[34]

The use of polyacrylamide gel electrophoresis in which gradient concentrations of the gels are used has been recommended for the fractionation of membrane glycoproteins,[35] which may be stained by an improved periodic acid–Schiff procedure.[36] Sulphated glycoproteins have been fractionated by isoelectric focusing on poly(tetrafluoroethylene)-coated columns;[37] this technique is likely to assist in studies of the acidic glycoproteins in mucins. N.m.r. spectroscopy, which is frequently applied to oligo- and poly-saccharides,[38] might be used more often in studies of glycopeptides.

Molecules in crystals of the trihydrated form of the model linkage compound 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine (D-Glc*N*Ac-Asn) adopt a $^4C_1$ conformation. The oxygen of the acetamido-group at C-2 is *cis* to the hydrogen atom at C-2, whereas the oxygen atom at C-1 is offset relative to H-1.[39] Moreover, the amido-group at C-1 is slightly non-planar, in agreement with the position of maximum stability calculated for peptide bonds generally.[40] The chiroptical properties of D-Glc*N*Ac-Asn in aqueous solution suggest that its stereochemistry is closely similar to that in the crystal.[41] The relative positions of the 2-acetamido-2-deoxy-D-glucose and aspartic acid moieties in the compound change quite markedly when the latter is complexed with trigonal hen egg-white lysozyme. The synthesis of such compounds as *O*-[2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-*N*-benzyloxycarbonyl-L-serine methylamide may permit further investigation into the stereochemistries of other linkage regions of glycoproteins.[42]

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The ability to cleave enzymically the di-*N*-acetylchitobiose structure adjoining glycosylated residues of asparagine in glycoproteins[43] and glycopeptides[44] has provided another procedure for the examination of glycoproteins. So far the enzyme has been found in cultures of *Streptococcus griseus*[43] and *Diplococcus pneumoniae*.[44] The products of acetolysis of glycopeptides may be analysed by g.l.c.[45]

Methods for coupling glycosylamines to the free carboxy-groups of proteins are likely to be used for synthesizing model glycoproteins.[46] Coupling has been carried out hitherto in the presence of either urea or guanidine, but use of the technique in the absence of a denaturing agent might furnish useful information.

Several general reviews relevant to glycoproteins have appeared,[47, 48] as well as more specific reviews which will be mentioned later. The synthesis of glycoproteins and the surface properties of mitochondrial membranes have also been considered.[49] It is of particular interest to examine whether or not glycoproteins on the surface of mammalian somatic cells are involved in the binding and penetration of sperm *in vitro*, as they are on the surface of the ovum.[50]

A number of glycoasparagines have been found in the urine of a patient with aspartylglycosaminuria,[51] and various aspects of the chemical pathology of glycoproteins continue to be of interest. The mannosidosis seen in Angus cattle has been attributed to a lack of the A- and B-forms of α-mannosidase.[52] Patients with two distinct forms of metachromatic dystrophy were found to have deficiencies of arylsulphatase A in their bone-marrow fibroblasts.[53] Other genetic defects are noted below. An increased urinary excretion of 2-acetamido-2-deoxy-β-D-hexosidase in diabetes has been reported.[54] The reason for this is unknown, but the amount correlates with the degree of glycaemia.

Electron microscopy requires staining of sections with phosphotungstic acid, but there are different views regarding its specificity. One report considers that the specificity at low pH resides, to a considerable extent, in sialic acid residues at least on the surface of ductal epithelial cells of human breast biopsies.[55]

Glycoproteins of Micro-organisms

The structure of the cell walls of bacteria has been studied extensively for a number of years, and the effects that antibiotics have on microbial walls and membranes have been discussed.[56]

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[56] M. R. J. Salton and A. Tomasz, *Ann. New York Acad. Sci.*, 1974, **235**, 6.

Lactobacillus bifidus was unable to use certain oligosaccharides as growth factors, unless sialic acid was first removed therefrom. This sugar moeity, especially in α-(2 → 6)-linkage, exerted a protective effect against *L. bifidus*.[57]

Lipids, possibly of the dolichol type, are considered to be involved in the transfer of D-mannosyl residues to endogenous glycoprotein acceptors in *Aspergillus niger*[58] and in the D-glucosylation of the O-34 antigen produced by group E_3 *Salmonella* carrying certain phages.[59]

The nature of the extracellular invertase produced by *Streptococcus mutans* and *S. salivarius* requires elucidation.[60] The major exocellular glycopeptide produced by *Penicillium charlesii* contains about ninety D-mannopyranosyl residues and a variable number of D-galactofuranosyl moieties, the latter being β-(1 → 5)-linked to each other. Some of the D-mannosyl residues are phosphorylated.[61] The carbohydrate moieties are attached through *O*-glycosidic linkages, involving D-mannose, to hydroxy-groups of either serine or threonine residues forming part of a polypeptide chain containing about thirty amino-acid residues.[62]

The structures of the O-specific side-chains in *Klebsiella* O Group 3 lipopolysaccharides are now more clearly understood; the repeating unit comprises five α-D-mannopyranosyl residues, two of which are (1 → 3)-linked and adjacent whereas the other three residues are (1 → 2)-linked.[63]

The formation of α-D-Gal*p*-(1 → 6)-D-Gal*p* and β-D-Gal*p*-(1 → ?)-D-Man*p* linkages, as part of the envelope glycoprotein, was catalysed by a particulate enzyme preparation from a fungus imperfectus (*Cryptococcus laurentii*).[64] The disaccharides formed part of dodecasaccharide side-chains linked to threonine and/or serine residues.

Glycoproteins present as cell-wall components of the fungus *Microsporum gypseum* probably have an important part to play in sporulation and spore germination.[65] The isolation, composition,[65a] and some of the chiroptical properties [65b] of the glycoprotein protease of *Mucor miehei* have been examined.

Mannan in the cell walls of a number of strains of yeast is linked to the protein backbone, mainly by linkages to the hydroxy-groups of serine and threonine residues.[66] Phosphorylated D-mannosyl residues are also present.[67]

Glycoproteins isolated from the cell walls of *Candida albicans* contain either mannans or glucans, the former attached to the protein by linkages that are labile to alkali (0.1M-NaOH, 48 hours, room temperature). In each case, the proteins are rich in threonine and serine.[67a]

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Dolichol monophosphate appears to be involved as an intermediate in the transfer of D-mannose from GDP-D-mannose to serine (threonine) residues, and to other acceptor sites also, in a specific glycoprotein in *Saccharomyces cerevisiae*.[68] However, there are also glycoprotein acceptors in yeast which will accept D-mannose from GDP-D-mannose without the involvement of a lipid intermediate, at least *in vitro*.[69] Of interest, particularly because of the general implications, is the finding that D-mannose appears to be essential for the synthesis of the protein chain of the glycoprotein acid phosphatase in a mannose-deficient mutant of *Schizosaccharomyces pombe*.[70] The evidence presented is not wholly definitive, since the results are capable of interpretation in other ways; the newly synthesized apoenzyme may be metabolically unstable unless it is glycosylated.

The mating factors that are responsible for the specificity of cellular recognition in the yeast *Hansenu wingei*, and which are glycoproteins, produced very large, polydisperse aggregates when they interacted.[71]

The type-specific antigens of avian and mammalian oncoviruses have been identified as glycoproteins, with the latter type, at least, possessing haem-agglutinating activity.[72] The envelope glycoproteins of mammalian oncogenic RNA viruses carry, perhaps not unexpectedly, multiple antigenic determinants.[73] It is of particular interest to note that two strains of parainfluenza virus-3 have equal haemagglutinating, but markedly different neuraminidase, activities. The anti-neuraminidase activity of nasal secretions against one of the varieties is IgA.[74]

In New Zealand mice affected with high tissue and serum levels of leukaemia virus, the virion envelope glycoprotein became selectively deposited in glomerular lesions.[75] The envelope glycoprotein comprises two closely related macromolecules of molecular weights 6.9×10^4 and 7.1×10^4.[73]

A mutant form of Rous sarcoma virus has been shown to lack glycoprotein, but the relation between the lack of the carbohydrate and the non-infectivity of the strain is unknown.[76] The terminal region of the carbohydrate moiety of the major glycoprotein of the Bryan strain of Rous sarcoma virus has been identified as α-D-Neu*N*Ac*p* → β-D-Gal*p* → β-D-Glc*N*Ac*p*.[77]

Glycosylation of the HA haemagglutinin of influenza-virus particles, when the virus infects cells, occurs in a stepwise fashion. Incorporation of 2-amino-2-deoxy-D-glucose occurs, in part, at the rough endoplasmic reticulum, whereas incorporation of L-fucose occurs at the smooth membrane.[78] The polypeptide precursor of glycoprotein HA appeared in cells treated with high concentrations of 2-amino-2-deoxy-D-glucose.[79]

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A study of the properties of the glycoproteins of Sendai virus has confirmed that in this paramyxovirus, as in many others, the haemagglutinin and neuraminidase activities are associated with a single glycoprotein.[80, 81] All Sendai virions appear to contain this glycoprotein, regardless of the nature of the cells in which the virus grows up.

Methods have been described for the purification of the glycoprotein (mol. wt. 3.8×10^4) from vaccinia virions.[82]

Serological characterization of a number of glycoproteins from mouse leukaemia virus has been reported.[83] The virus virion, when grown up in primary cultures of BALB/c or C3H mouse mammary tumour cells, contains at least five glycoproteins.[84] It will be of interest to compare these glycoproteins with those present in a virus sometimes present in the mother's milk.

The spike glycoproteins of Semliki Forest virus appear to extend throughout the viral membrane and to come into close contact with the nucleocapsid.[85] The spike glycoproteins appear to arrive at the host-cell plasma membrane before the nucleocapsid,[86] and, by penetrating the membrane, may act as a site of attraction for the nucleocapsid itself.[85]

Vesicular stomatitis virus contains a membrane glycoprotein whose quantitative carbohydrate composition (Fuc, Neu*N*Ac, Gal, Gal*N*Ac, Man, and Glc*N*Ac) appears to be largely independent of the type of cell line in which it has been grown up.[87]

Cells infected with an Arborvirus synthesized glycoprotein more rapidly than their normal forms, particularly during virus multiplication.[88]

Japanese encephalitis virus has been shown to contain two structural membrane glycoproteins, one of which is known to be modified during morphogenesis; 2-amino-2-deoxy-D-glucose and D-galactose are among the sugars present.[89]

The glycoproteins of measles virus form part of the surface projection and are responsible for the haemagglutinating, haemolytic, and cell-fusion activities; the last two activities also require the presence of certain membrane lipids.[90]

The surface of avian myelomatosis virion appears to contain a glycolipoprotein which is antigenic in guinea-pigs that have been immunized intracerebally.[91] The determination of the precise nature of the antigen is awaited with considerable interest.

The tomato spotted wilt virus contains three glycoproteins (mol. wts. 8.4×10^4, 5×10^4, and 2.9×10^4) that constitute 98% of the total viral protein.[92]

The inhibitory effects of 2-deoxy-D-*arabino*-hexose on the production of virus glycoproteins and on the glycosylation of immunoglobulins may operate through

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[92] N. A. Mohamed, J. W. Randles, and R. I. B. Francki, *Virology*, 1973, **56**, 12.

the mediation of activated intermediates, which include UDP- and GDP-2-deoxy-D-*arabino*-hexose.[93]

Human interferon, a glycoprotein of molecular weight 2.5×10^4, can be isolated from leucocytes and foreskin diploid fibroblast extracts by immunoadsorbent affinity chromatography.[94] Anti-interferon, induced against interferon from leucocytes, was used as the antibody. Albumin immobilized in agarose selectively bound human interferon, whereas mouse interferon was only weakly bound, and rabbit interferon not at all.[95] Albumin from man, mouse, or cattle was equally effective. Further work is needed to establish whether or not these differences are due to different sugar levels in the various interferons. Although rabbit interferon has non-reducing terminal units composed of α-Neu*N*Ac*p* → β-D-Gal*p*,[96] the carbohydrate moiety does not appear to have a role in the turnover of circulatory human-leucocyte interferon in rabbits.[97]

Higher Plant Glycoproteins

Plant lectins, many of which are glycoproteins, will be considered with lectins in general. In view of the presence of lectins in a number of plant cell walls, it has been suggested that they bind to other glycoproteins, thereby becoming involved in the regulation of plant-wall extension.[98] A glycopeptide containing 2-amino-2-deoxyglucose, mannose, galactose, fucose, and xylose has been isolated from a preparation of ficin.[99] The carbohydrate–peptide linkage thereof would be expected to involve an asparaginyl residue. An arabinogalactan–peptide isolated from wheat endosperm was shown to have a molecular weight of 2.2×10^4, to contain α-L-arabinofuranosyl and, probably, β-D-galactopyranosyl residues, and to consist of 8% by weight of a peptide having all of its hydroxyproline residues glycosylated.[100, 101] The coat proteins of two seed-transmitted viruses, *viz.* barley stripe mosaic virus and cowpea mosaic virus, consist largely of glycoproteins, whereas those of three plant viruses not transmitted by seeds do not contain carbohydrate.[102] It has been suggested that the glycoproteinaceous nature of the capsid might determine the transmissibility of a virus in seeds.

The relation of the structure to the function of several phytotoxic glycopeptides and other glycosides from plant pathogens has been considered.[103]

The structures and characteristics of some glycoproteins of algae of the genus *Phyllophora* have been explored,[104] as have glycosphingolipids and glycoproteins

[93] M. F. G. Schmidt, R. T. Schwarz, and C. Scholtissek, *European J. Biochem.*, 1974, **49**, 237.
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in wild type and in non-aggregating mutants of *Dictyostelium discoideum*.[105] Mutations affecting 2-acetamido-2-deoxy-β-D-glucosidase have been induced in the latter species.[106]

One of the glycosyltransferases in the Golgi membranes of onion stems has properties similar to the lactose synthetase:α-lactalbumin system.[107]

Commercial horse-radish peroxidase is often found to contain a potent, immunogenic glycoprotein possessing no peroxidase activity.[108]

Lectins

Lectins have been used increasingly in studies of a variety of biological phenomena, including the study of membranes.[109] Carbohydrates on cell membranes can be detected using lectin–peroxidase conjugates,[110] although it is questionable whether such techniques will help to locate the normal positions of the carbohydrates precisely, since, for example, it is known that mitogenic lectins induce changes in the fluidity of lymphocyte membranes.[111] Other evidence for the induced mobility of the binding sites of lectins on cells, in particular of concanavalin A on lymphocytes, malignant lymphoma cells, and normal and transformed Simian virus cells, was provided by the results of studies of rotational relaxation times using fluorescent probes.[112] Staining of cells, already fixed with glutaraldehyde, with a complex of iron dextran–concanavalin A may be useful in future investigations of the problem of fluidity.[113]

That the relative mobilities of agglutinin receptor sites on the plasma membranes of rabbit spermatozoa are greatest on the postacrosomal region was demonstrated by the use of ferritin-labelled agglutinins from *Ricinus communis*.[114] Lectins were also used in studies which suggested that complex saccharide moieties are randomly distributed on the surface of the parasitic protozoan *Leishmania donovani*.[115] The innermost, jelly-coat layer of the ova of *Xenopus laevis* binds wheat-germ agglutinin and concanavalin A, but it is not clear whether or not the sugar components of this layer assist in preventing polyspermy.[116]

The clustering of intramembranous particles that precedes the agglutination of cells exposed to concanavalin A appeared to be associated with the ability to agglutinate and not to be due directly to binding of the lectin.[117] This phenomenon was demonstrated with plasmacytoma cells, but may be general.

A wide-ranging discussion on the biomedical properties of agglutinins has stressed the properties of lectins from invertebrates, as well as those of lectins

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from plants. Interactions of different lectins with erythrocytes, leucocytes, cell-bound antibodies, and blood-group substances, as well as the mechanisms of the interactions, have been discussed.[118]

The toxicity of lectins is a matter of more than academic concern, since beans are sometimes cooked inadequately without destroying the toxic properties of the lectins.[119] Other aspects of this problem have been discussed.[120]

A method for quantitatively measuring the agglutination of BALB/c 3T3 cells induced by concanavalin A is likely to be of general application.[121] Agglutination appeared to be more complex than simply binding of the cells by a bridging lectin.

The effects of temperature on plasma-membrane acceptor sites and on agglutination have often been studied using lectins.[122] The results of these experiments require careful interpretation, at least when concanavalin A is used; this lectin readily dissociates to the dimeric form as the temperature is reduced from 37 °C, and the agglutinating abilities of the dimeric and tetrameric forms differ.[123, 124]

Lectins are known to exert effects on whole animals, as was implied above. Highly purified concanavalin A and other lectins produced intense oedema when injected into the footpads of animals, but the reason for this is not clear.[125]

Immunosuppression occurred in mice injected intraperitoneally with concanavalin A two days before primary immunization with a thymus-dependent sheep red-cell antigen, but not with a thymus-dependent lipopolysaccharide antigen from *Escherichia coli*.[126]

The use of lectins in the isolation of glycoproteins from synaptic junctions of brain [127, 128] is likely to assist investigation of the carbohydrates in this organelle.[129]

The functions of lectins *in vivo* are still obscure. It has been suggested that the symbiotic nitrogen-fixing relationship, involving root-nodule formation on soybeans and legumes by soil bacteria of the genus *Rhizobium*, is initiated through interactions involving plant lectins and bacterial saccharides.[130]

The plasma membranes of cells usually carry binding sites for lectins. Plasma membranes from rat liver and mammary gland bind concanavalin A in a manner predicted from studies on whole cells.[131] Milk-fat globule membranes exhibited a high capacity to bind the lectin.

A selected population of liver cells can be obtained by utilizing the specific ability of the plasma membranes to bind concanavalin A; these cells appeared to have a greater rate of glycogenesis than the remainder of the cells.[132] The use of

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lectins may thus provide a valuable tool for separating populations of hepatocytes with different functions.

Red-cell surfaces are particularly rich in binding sites for lectins. Competitive binding between group O erythrocytes and lectins from a variety of sources suggested that *N*- and *O*-glycosidically-linked carbohydrate moieties could be differentiated by this technique.[133] However, highly standardized procedures must be used to examine haemagglutination induced by lectins, since, for example, the rate of haemagglutination induced by concanavalin A is reduced as the temperature is lowered, whereas that induced by soybean agglutinin is increased.[134]

Glycoproteins that carry receptor sites for wheat-germ agglutinin, *viz. Ricinus communis* lectins,[135] concanavalin A, and *Lens culinaris* lectin,[136] have been isolated from human red-cell ghosts by affinity chromatography.

As implied above, the events involved in the induction of lymphocyte proliferation by mitogens are not well understood. The binding of concanavalin A to horse lymphocytes was followed by the binding of cellular, histone-free proteins to chromatin and by the phosphorylation of specific, nuclear, acidic proteins, probably prior to extensive gene activation.[137]

Concanavalin A and phytohaemagglutinin bind to, and are distributed equally well on, B- and T-lymphocytes.[138] The studies suggested that capping and the triggering of blastogenesis are not necessarily directly related phenomena. However, the effects of concanavalin A on the response of B-lymphocytes to antigens *in vitro* depended on the presence of T-cells.[139]

The carriers of θ, TL, and H-2 alloantigens on the surface of mouse lymphocytes, as well as the receptors for concanavalin A, are mobile in the plane of the membrane. All the membrane macromolecules are potentially mobile, and uneven distribution is probably induced by the ligands used to examine them.[140] The histocompatibility antigens on circulatory lymphocytes are probably distinct from those carrying the binding sites for lectins.[141]

The effects of treatment with glycosidases on the subsequent ability of peripheral lymphocytes to undergo transformations induced by concanavalin A have been studied.[142]

Mitogens, in particular phytohaemagglutinin and concanavalin A, stimulate rosette formation of human lymphocytes.[143] There is a parallelism between this effect and blastogenesis induced by the lectins.

Bone-marrow cells from mice responded *in vitro* more weakly to concanavalin A and to phytohaemagglutinin than did cells from the spleen.[144] The relative responses of the two sets of cells differed.

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The toxicity of rubidomycin, an antibiotic of the anthracycline group used in the treatment of leukaemia, was increased by pokeweed mitogen, but not by phytohaemagglutinin.[145] The reason for this is not clear.

Extensive bonding of concanavalin A to the nuclear membrane of calf thymocytes indicated that the number of binding sites per unit area is closely similar to that of the plasma membrane.[146]

Carbohydrates on the surfaces of bacterial cells provide sites of interaction for lectins. The lectins from *Pisum sativum* and *Lathyrus sativus* were indicated to bind to the same receptor sites as does the competence substance in the cell walls of pneumococci.[147] The implication of this finding requires further study, especially as 2-amino-2-deoxy-D-glucosyl and -D-galactosyl residues, rather than the *N*-acetylated sugars, seem to form part of the determinant site recognized by competence substance. The mechanism of binding of the latter has been discussed.[148]

Concanavalin A inhibits the adsorption of bacteriophage to the D-glucosylated teichoic acid of *Bacillus subtilis* 168, and the lectin can be used to locate teichoic acids in cell walls.[149] The precise conditions needed for these interactions have been described.

Lectins present in invertebrates have been examined further. Limulin, a sialic-acid-binding lectin from the horse-shoe crab *Limulus polyphemus*, has been found to have a molecular weight of 3.35×10^5, comprising subunits of molecular weight 1.9×10^4, and an isoelectric point of 5.1.[150] It is a glycoprotein.

Two agglutinins in the haemolymph of the lobster *Homarus americanus* have sedimentation coefficients of 11*S* and >19*S*, respectively. Each requires Ca^{2+} ions for activity and dissociates into subunits (mol. wt. 5.5×10^4) in 6M-urea.[151] The larger molecule exhibits an affinity for sialic acid residues, whereas that of the smaller molecule is for 2-acetamido-2-deoxy-D-galactosyl residues.[152]

For the anti-H agglutinin of eel serum (mol. wt. 1.4×10^5) there are about $1.7—1.9 \times 10^6$ binding sites on human group O erythrocytes and about 0.4×10^6 on human peripheral lymphocytes.[153] Anti-H agglutinin has also been isolated from the seeds of *Cytisus sessilifolius*. The anti-A agglutinin isolated from the albumin gland of the snail *Euhadra callizona amaliae* has a molecular weight of 8.9×10^4, an isoelectric point of 3.6, and contains 5.1% hexose.[154]

Lectins from mature ova of the fish *Salmo trutta*, *Clupea harengus*, and *Rutilus rutilus* exhibited high affinities for D-galactosyl and L-rhamnosyl residues, and bound selectively to blood-group B substance and the P_1 substance from sheep hydatid cyst fluids.[155]

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Orthorhombic crystals of an L-arabinose-binding protein from *E. coli* contain asymmetric molecular units, comprising one protein molecule of molecular weight 3.8×10^4.[156]

Attempts to relate the toxicities of ricin and abrin to their abilities to interact with cells have furnished valuable information. The binding to, and entry of, abrin and ricin into Ehrlich ascites cells were studied as part of an examination of the toxicities of the plant extracts.[157] These non-agglutinating toxins are closely similar to, but distinct from, agglutinins (mol. wts. 1.84×10^5 and 1.2×10^5, respectively) found in *Abrus precatorius* and *Ricinus communis*, which have been extensively purified.[158] The relation between the structures of the toxins and the agglutinins is of considerable interest, since all bind D-galactosyl residues. There are two lectins in the castor bean (*R. communis*), with molecular weights of approximately 1.2×10^5 and 6.5×10^4, having structures that can be represented by $\alpha_2\beta_2$ and $\alpha\beta^1$, respectively.[159, 160] The binding sites for the lectins on a mouse lymphoma were not identical.[161] Amyloids from tamarind, balsamine, and capucine also precipitate with at least one of the *R. communis* lectins, suggesting that the structures of the amyloids might have more interesting features than previously recognized.[162]

Ricin is probably the fifth most toxic polypeptide known in higher animals, after tetanus, botulinus, and diphtheria toxins, and gramicidin.[163] It is not known whether or not its toxicity is related to the ability to induce drug-metabolizing enzymes in the livers of rats injected with the lectin.[164]

The structure of wheat-germ agglutinin has become clearer. The agglutinin has been isolated from wheat germ by affinity chromatography on ground chitin, and has been shown to have a blocked *N*-terminus,[165] in agreement with earlier studies.[166] The lectin has two independent and homogeneous binding sites for reduced chitotetraose [167] and for 4-methylumbelliferyl glycosides.[168] Fluorescence studies showed that at least one of the sites involves tryptophanyl residues.[169] Crystallographic examination of wheat-germ agglutinin has been initiated.[170]

Wheat-germ agglutinin is able to distinguish a group of leucocytes, obtained from the spleens of BALB/c mice, which carry neither immunoglobulin nor T-cell markers.[171]

Concanavalin A has continued to be studied extensively. The sugar-binding site of this lectin may be usefully examined by spectrophotometry.[172]

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The ability of concanavalin A to bind non-polar molecules also means that care must be exercised in interpreting the results of binding experiments utilizing, for example, cell membranes.[173] The different binding orientation of methyl α- and β-D-glucopyranosides, as revealed by *X*-ray crystallographic studies, probably accounts for the differences between the binding constants of the two glycosides.[174, 175] Sugars bound to concanavalin A provided that the S_1-site was occupied by a transition-metal ion.[176]

Concanavalin A binds glycopeptides from hen-egg albumin more strongly than it does the entire glycoprotein.[177] It inhibits the enzymic activity of thrombin *in vitro* by binding to a carbohydrate moiety on the enzyme, providing at least one way whereby the lectin is able to prolong the thrombin clotting-time of human plasma.[178]

The introduction of maleyl groups into concanavalin A *in vitro* led to the formation of dimeric species from the tetramer; at least eight such groups must be introduced to achieve complete dissociation into the dimer, which retains the ability to bind carbohydrate.[179]

The structure of hexagonal crystals of concanavalin B (mol. wt. 3.0×10^4) has been studied by *X*-ray crystallography.[180]

Lens culinaris haemagglutinin is able to bind glycopeptides from transferrin and IgM, but not those from egg albumin.[177]

It is not surprising that affinity chromatography has been so extensively used in the isolation of lectins. Formalinized erythrocytes have been used in the separation of agglutinins from jack-bean meal, wheat germ, seeds of European gorse (*Ulex europaeus*), and lima beans.[180a] Wheat-germ agglutinin has also been isolated using a thioglycoside of 2-acetamido-2-deoxy-D-glucose linked to Sepharose as an adsorbent.[180b] This procedure can be used for the isolation of glycosidases. Coupling of reduced *p*-nitrophenyl glycosides with Sepharose has led to a variety of supports for use in the affinity chromatography of various lectins.[180c] Another valuable technique involves electrophoresis of partly purified extracts of plants on *O*-glycosylated polyacrylamide gels; this procedure is likely to be of general applicability.[181]

Concanavalin A has been isolated (by affinity chromatography) from two species of *Canavalia* (*C. ensiformis* and *C. gladiate*); the proteins are closely similar.[182]

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Affinity chromatography in which the lectin is immobilized has also been used extensively. Procedures using concanavalin A have been employed in purifying mycobacterial polysaccharides,[183] grass pollens,[184] human-plasma lipoproteins,[185] dopamine β-hydroxylase from bovine adrenal glands,[186, 187] and exonuclease from venom of the rattle-snake *Crotalus adamanteus*.[188] The purification procedure also provides an indication of the structures of the carbohydrate moieties of these glycoproteins. The binding to, and precipitation by, concanavalin A of arylsulphatase A, acid phosphatase, 2-acetamido-2-deoxy-β-hexosidase, β-galactosidase, and β-glucuronidase of sheep-brain lysosomes were used in purification of the enzymes.[189]

Blood-group Substances

The biochemical basis for human ABO and Lewis blood groups has been discussed in terms of the structures of the oligosaccharides of the secreted glycoproteins.[190] Biochemical genetics have shown that the locus for the MN blood group is probably near the centromere of the long arm of chromosome no. 2.[191]

A rare type of antibody found in the serum of a subject reacted only with red cells having both A and Le^b characters. H Character could be demonstrated in either saliva or on red cells.[192] The biochemical genetics of this unusual condition were discussed. A new, rare blood-group antigen, termed 'FAR', has been described; it is inherited as a Mendelian dominant character and is linked to the MNSs system.[193]

The Le^x determinant is distinct from Le^a and Le^b. It has been suggested that it is produced by the action of an α-fucosyltransferase on type II precursor, whereas Le^a is produced by the action of this enzyme on type I precursor.[194] Chemical methods could be used to test the validity of this suggestion. Of considerable interest is the finding that treatment of the red cells of an individual having the Bombay blood group with neuraminidase led to the appearance of groups reacting with anti-A_{Hp} (*Helix pomatia*), which is usually considered to be an A-binding antibody although it does bind other specific antibodies.[195] The nature of the determinant sites was not assessed in chemical terms.

IgM anti-A and anti-B antibodies were found to bind more strongly to A and B glycoproteins, respectively, than did the corresponding IgG antibodies. These findings may explain why A and B glycoproteins strongly inhibit the agglutination of red cells by IgM anti-A and anti-B antibodies, respectively, but only do so weakly when IgG antibodies are used.[196]

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A number of reduced oligosaccharides (2)—(19), many of previously unknown structure, have been prepared by treatment of two blood-group substances with alkaline borohydride.[197] Each of the structures exhibited the anticipated biological activity.[198]

β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
D-Gal*N*Acol = 2-acetamido-2-deoxy-D-galactitol
(2)

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

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

α-L-Fuc*p* β-D-Gal*p*
1 1
↓ ↓
4 3
β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol
(5)

α-L-Fuc*p* β-D-Gal*p*
1 1
↓ ↓
4 3
β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol
(6)

β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*
1
↓
6
β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
6
↑
1
β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*
(7)

α-L-Fuc*p* β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*
1 1
↓ ↓
4 6
β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
6
↑
1
β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*
(8)

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α-L-Fuc*p*
1
↓
4
β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
6
↑
1
β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*

(9)

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

(10)

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

(11)

α-L-Fuc*p* β-D-Gal*p*
1 1
↓ ↓
3 3
β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol

(12)

β-D-Gal*p*
1
↓
3
α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol

(13)

α-L-Fuc*p* β-D-Gal*p*
1 1
↓ ↓
3 3
α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol

(14)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol
3
↑
1
α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*

(15)

α-L-Fuc*p*
1
↓
3
α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 6)-D-Gal*N*Acol
3
↑
1
α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*

(16)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol

(17)

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

(18)

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

(19)

Certain gastric tumours have been shown to carry blood-group A- and B-like activities which, surprisingly, were found to be independent of one another in certain areas of the malignant growth.[199]

Lipids extracted from pig stomach mucosa contained a ceramide tetrasaccharide (20), which exhibited blood-group H activity but lacked a 2-acetamido-2-deoxy-D-glucosyl residue.[200] The presence of an α-D-galactosyl residue in this substance is unexpected. An A-active ceramide hexasaccharide (21) lacking 2-acetamido-2-deoxy-D-glucose is also a minor component of pig stomach mucosa.[201] The structures of L and U glycolipids from pig gastric mucosa are depicted in (22) and (23), respectively.[202] A ceramide tetrasaccharide from human red cells has the structure (24).[203]

Guinea-pig spleen contains a 2-acetamido-2-deoxy-α-D-galactosyltransferase that catalyses the conversion of kidney globoside into the Forsmann antigen, one of the most potent haptenic sphingoglycolipids.[204]

[199] H. Denk, G. Tappeiner, and J. N. Holzner, *Nature*, 1974, **248**, 428.
[200] B. L. Slomiany, A. Slomiany, and M. I. Horowitz, *European J. Biochem.*, 1974, **43**, 161.
[201] B. L. Slomiany, A. Slomiany, and M. I. Horowitz, *Biochim. Biophys. Acta*, 1973, **326**, 224.
[202] A. Slomiany, B. L. Slomiany, and M. I. Horowitz, *J. Biol. Chem.*, 1974, **249**, 1225.
[203] B. Siddiqui and S.-I. Hakomori, *Biochim. Biophys. Acta*, 1973, **330**, 147.
[204] S. Kijimoto, T. Ishibashi, and A. Makita, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 177.

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

(20)

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

(21)

α-D-Gal*NAcp*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Glc*NAcp*-(1 → 3)-β-D-Gal*p*-
(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(22)

α-D-Gal*NAcp*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*NAcp*-(1 → 3)-β-D-Gal*p*-
(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(23)

β-D-Gal*p*-(1 → 4)-β-D-Glc*NAcp*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide

(24)

Amino-acid sequences present in peptide chains in regions carrying the carbohydrate moieties of soluble blood-group substances from ovarian cysts include Thr-Thr-Ser, Thr-Ser-Thr-Ser, Thr-Pro-Thr-Ser-Ser, Pro-Thr-Thr-Thr-Pro-Ser, and Ala-Pro-Thr-Thr-Ser-Gly-Ser, but it is not known precisely which serine and/or threonine residues are glycosylated.[205]

The glycopeptides Ser-α-D-Gal*NAcp*, Pro-Ser-D-Gal*NAcp*, Thr-Ala-Ser-D-Gal*NAcp*-(3 → 1)-D-Gal*NAcp*, and Asn-D-Glc*NAcp*, were isolated from among the degradation products of glycoproteins from pig-stomach linings.[206]

The relative contributions of glycolipids and glycoproteins to the blood-group activity of red cells presents an interesting challenge. It has been suggested that the H-antigenicity of erythrocytes resides only on glycolipids; this was deduced from the fact that the H-activity of glycoproteins (isolated from red cells by use of lithium di-iodosalicylate) was lost when the preparations were chromatographed on phytohaemagglutinin–Sepharose 2B.[207]

Cross-reacting substances with H-activity have been found in a number of invertebrate extracts. Catfish and eel anti-H sera precipitated with galactogens present in albumin-gland extracts from five species of snails, although the galactogens do not appear to contain L-fucose.[208] The possible nature of the determinants was discussed.[209] The agglutinin specificities and blood-group H-like activities in extracts of the molluscs *Pomacea paludosa* and *P. urceus* have been studied.[210]

[205] S. D. Goodwin and W. M. Watkins, *European J. Biochem.*, 1974, **47**, 371.
[206] N. K. Kochetkov, V. A. Derevitskaya, L. M. Likhosherstov, and S. A. Medvedev, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 311.
[207] B. A. Brennessel and J. Goldstein, *Vox Sanguinis*, 1974, **26**, 405.
[208] B. A. Baldo, G. Uhlenbruck, and G. Steinhausen, *Vox Sanguinis*, 1973, **25**, 398.
[209] B. A. Baldo and G. Uhlenbruck, *Immunology*, 1973, **25**, 649.
[210] B. A. Baldo and G. Uhlenbruck, *Vox Sanguinis*, 1974, **27**, 67.

Digestion of human ovarian cyst A substance with elastase coupled to Sepharose 2B furnished a series of peptides and glycopeptides which could be separated by affinity chromatography using *Vicia cracca* lectin. The results showed that about 70% of the serine and threonine residues are glycosylated, and also confirmed that non-glycosylated regions exist in the polypeptide chains.[211] Undegraded A substance from a single individual has been separated, by chromatography on *Vicia cracca* lectin–Sepharose 2B, into subfractions differing in amino-acid and sugar compositions.[212] The physical properties of subfractions of the blood-group substances also pointed to the existence of molecules having different peptide components.[213]

Blood-group A substance coupled to agarose is of value as an immunoadsorbent.[214]

The distribution of blood-group antigens in butanolic extracts of human erythrocyte 'ghosts' has been carefully re-examined.[215] It was found that there is no correlation between the secretor status of the donor and the presence of blood-group A activity in the aqueous phase under any of the conditions used for extraction. This is contrary to prevailing opinion.

A proportion of the red cells of patients with the Tn-syndrome exhibit decreased electrophoretic mobility and sialic acid content, but possess additional A-like determinants.[216] The sites responsible for these properties on the red cells of the patients probably involve 2-acetamido-2-deoxy-α-D-galactosyl residues directly linked to the polypeptide chain; these residues normally occur as internal regions of the MN determinants.

The (anti-A_1) snail agglutinin from the albumin gland of *Euhadra callizona amaliae* has a molecular weight of 8.9×10^4 and contains 5.1% hexose.[217] Its haemagglutinating activity was inhibited by 2-acetamido-2-deoxy-D-glucose, raffinose, and melibiose, as well as by 2-acetamido-2-deoxy-D-galactose.

Blood-group A-active substances were found in extracts of *Amphioxus* (*Branchiostoma*) *lanceolatus* and in the sera of *Phallusia mammilata*, which are lower chordates generally considered to be the connecting link between vertebrates and invertebrates.[218] Serological tests suggested that the A-activity is due to the presence of non-reducing, terminal 2-acetamido-2-deoxy-α-D-galactosyl residues.

The properties of enzymes used *in vitro* for interconversion of the blood-group substances are not adequately known. α-D-Galactosidase from coffee beans, which is of value in studies of B substance, can be conveniently purified by affinity chromatography.[219]

The serum of a healthy patient with A_1H specificity on her red cells and in her saliva had no anti-B activity, nor was the production thereof stimulated by

[211] T. Kristiansen, *Biochim. Biophys. Acta*, 1974, **338**, 374.
[212] T. Kristiansen, *Biochim. Biophys. Acta*, 1974, **338**, 246.
[213] J. M. Creeth, K. R. Bhaskar, A. S. R. Donald, and W. T. J. Morgan, *Biochem. J.*, 1974, **143**, 159.
[214] T. Kristiansen, *Biochim. Biophys. Acta*, 1974, **362**, 567.
[215] D. J. Anstee and M. J. Tanner, *Biochem. J.*, 1974, **138**, 381.
[216] W. Dahr, G. Uhlenbruck, and G. W. G. Bird, *Vox Sanguinis*, 1974, **27**, 29.
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[219] N. Harpaz, H. M. Flowers, and N. Sharon, *Biochim. Biophys. Acta*, 1974, **341**, 213.

immunization with human blood-group B glycoprotein.[220] This unusual finding might have important implications.

An unusual case has been reported of four related individuals of Finnish descent having a weak B antigen on their erythrocytes and with no B substance in their saliva, but with anti-B antibodies present in their sera.[221]

The molecular weight of the major glycoprotein from human erythrocyte membrane has been reinvestigated; the value (2.9×10^4) found [222] is closely similar to that originally reported by Winzler.

The ABO-grouping of hair has been achieved by the use of ^{125}I-labelled anti-A and anti-B sera and extracts of *Dolichos biflorus*.[223]

Collagens

A rapid, automatic, chromatographic procedure has become available for the separation of hydroxylysine and glycosides thereof from alkaline hydrolysates of collagen and basement membrane.[224] Enhanced excretions of galactosyl-hydroxylysine have been noted in the urine of patients with bone diseases and of glucosylgalactosylhydroxylysine in the urine of those with skin diseases.[225]

Several of the hydroxylysine residues of invertebrate collagens, such as sea anemone (*Actinia equina*), liver fluke (*Fasciola hepatica*), and snail (*Helix pomatia*), are substituted by glucosylgalactosyl moieties, just as they are in the vertebrate collagens.[226] The collagens from sea anemone contain at least 70% of hydroxylysine in glycosylated form.[227] In the body-wall collagen of the sea cucumber (*Thyone briareus*), the glycosylated residues become involved in cross-linking processes as maturation occurs.[228]

Glucosylgalactosylhydroxylysine isolated from digests of bovine-aorta collagen can be cleaved by an α-D-glucosidase, but not by a β-D-glucosidase, present in aorta. The organ also contains α- and β-D-galactosidases, but only the latter cleaved galactosylhydroxylysine.[229] Cross-linked peptides have been isolated from collagen by means of affinity chromatography in which antibodies to collagen were used; glycosylated and uncharged peptides were separated by this technique.[230]

Denatured α_1-chains from chicken-skin collagen caused human platelets to aggregate. A glycopeptide [of partial structure (25)] isolated from this collagen inhibited the interaction.[231] The carbohydrate portion appeared to be involved.

...Glc-Gal-*O*-Hyl-Gly-His-Arg-Gly-Phe-Ser-Gly...

(25)

[220] G. F. Springer and H. Tegtmeyer, *Vox Sanguinis*, 1974, **26**, 247.
[221] S. K. Zelenski, B. Litsenberger, and R. H. Aster, *Vox Sanguinis*, 1974, **26**, 189.
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[229] G. Pott, W. Henkel, and E. Werries, *Z. physiol. Chem.*, 1974, **355**, 787.
[230] J. W. Chidlow, F. J. Bourne, and A. J. Bailey, *Immunology*, 1974, **27**, 665.
[231] A. H. Kang, E. H. Beachey, and R. L. Katzman, *J. Biol. Chem.*, 1974, **249**, 1054.

The stimulus given by bacterial lipopolysaccharides to the production of collagenase by macrophages is relatively specific, but the effects of these lipopolysaccharides on the cell membrane remain to be examined.[232]

Procollagen is probably disulphide bridged but, as with other disulphide-bridged glycoproteins, it is not clear whether bridge formation or glycosylation occurs first.[233]

The presence of its degradation products in urine is a measure of the rate of breakdown and of synthesis of collagen by the body. The levels of four enzymes involved in post-translational modifications of collagen have been assessed in the livers of rats into which carbon tetrachloride was injected twice weekly.[234] Within two weeks of beginning the injections, the prolyl- and lysyl-hydroxylase levels, as well as those of the collagen galactosyl- and glucosyl-transferase activities, had increased, whereas the collagen levels were unchanged. The changes are therefore not due to an increase in the number of collagen-forming cells. The levels of glycosylating enzymes in organs of mice with a sex-linked defect at the Mottled locus are unknown.[235] There is likely to be a change in these levels, compared with normals, since the Mottled mice failed to make allysine residues, some of which are usually glycosylated, for cross-linking.

^{18}O-Labelling techniques have yielded results suggesting that the greater part of the collagen deposited in granulation tissue is derived from proteolysis of preformed collagen.[236] The products of such proteolysis are used very efficiently, but the fate of the sugars is unknown.

The cause of thickening of the glomerular basement membrane in the kidneys of diabetics has been reported not to be due to increased hydroxylation and glycosylation of lysine residues.[237, 238]

Chondroitin 4-sulphate, dermatan sulphate, hyaluronic acid, and keratan sulphate each interact with calf-skin collagen in solution at pH 4; these interactions stabilize the triple-helical structure of the collagen.[239, 240] The difficulties in extracting glycosaminoglycans from cartilage with salts are in part due to interactions of this type. If the concentration of calcium chloride used is too high, collagen is denatured and the amount of polysaccharide taken into solution decreases.[241]

Sialic-acid-containing glycoconjugates, probably on the plasma membrane of fibroblasts, have some sort of role in the production of collagen by the cells. Thus, slices from experimental granuloma or free embryonal fibroblasts lost the capacity to incorporate [^{3}H]proline into collagen.[242] There was little or no change in the rates of formation of non-collageneous proteins and nucleic acids by the cells.

[232] L. M. Wahl, S. M. Wahl, S. E. Mergenhagen, and G. R. Martin, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 3598.
[233] J. M. Monson and P. Bornstein, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 3521.
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[238] N. A. Kefalides, *J. Clin. Invest.*, 1974, **53**, 403.
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[240] R. A. Gelman and J. Blackwell, *Connective Tissue Res.*, 1973, **2**, 31.
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[242] M. Aalto, T. Rönnemaa, and E. Kulonen, *Biochim. Biophys. Acta*, 1974, **342**, 247.

The cross-linkages of guinea-pig skin collagen were found to be relatively labile to heat, being cleaved by heating at 80 °C and pH 7.5 within 3 hours.[243] The cross-linkages of scar collagen were shown to be more stable, but it is not clear whether the carbohydrate portion fulfils a stabilizing role. The cross-linkages in granuloma and embryonic skin collagens are similar, but there are some differences from those present in normal skin collagen.[244]

Collagens of the type represented by $[\alpha 1(III)]_3$ occur in human skin, aorta, and uterine leiomyoma.[245] Differences from the predominating type of collagen ($[\alpha 1(I)_2\alpha 2]$) in these tissues are not due solely to the degree of glycosylation of hydroxylysine residues.

Glycogens

Three methods of analysis for glycogen in tissue homogenates, *viz.* (i) acid hydrolysis followed by enzymic assay for D-glucose, (ii) enzymic hydrolysis with amylo-α-1,4:α-1,6-glucosidase followed by enzymic assay for D-glucose, and (iii) degradation with phosphorylase and a debranching complex followed by assay for D-glucose 1-phosphate, yielded closely similar values for most tissues.[246] Brain gave artificially high values if acid hydrolysis was used. The second of the foregoing procedures was also strongly recommended by other workers.[247]

Shellfish glycogen has been confirmed to have regions of dense branching that are resistant to the action of α-amylase; these areas appear to be randomly distributed throughout the molecule.[248] Now that paracrystalline glycogen is available, it should be possible to examine these aspects in greater detail by physical methods.[249]

The ability to culture pancreatic β-islet cells has provided a means of examining the effects of D-glucose thereon.[250] Tissues cultured in media containing relatively high levels of D-glucose (up to 28 mmol l^{-1}) contained elevated levels of glycogen, ATP, and the form of hexokinase with a high K_m value.[251] The metabolism of glycogen has also been studied in isolated rat livers perfused with D-fructose and sorbitol.[252] Glycogenolysis, already relatively rapid,[252] was increased in rat livers perfused with either glucagon or a mixture of iodoacetate (1 mmol l^{-1}) and cyanide (1 mmol l^{-1}).[253] The liver of the conger eel *Amphiuma means* could prove of considerable use in experimental studies, since the glycogen of fragments thereof maintained in culture was not degraded extensively over a period of 35 days.[254] The glycogen content fell when epinephrine was added to the medium, but it increased in the presence of either insulin or additional D-glucose.

[243] D. S. Jackson, S. Ayad, and G. Mechanie, *Biochim. Biophys. Acta*, 1974, **336**, 100.
[244] A. J. Bailey, S. Bazin, and A. Delaunay, *Biochim. Biophys. Acta*, 1973, **328**, 383.
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[247] K. L. Roehrig and J. B. Allred, *Analyt. Biochem.*, 1974, **58**, 414.
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[249] H. De Wulf and H. G. Hers, in ref. 103, p. 399.
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[251] A. Andersson, E. Borglund, and S. Brolin, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 1045.
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[253] A. Jakob and S. Diem, *Biochim. Biophys. Acta*, 1974, **362**, 469.
[254] M. A. Monnickendam, D. Brown, and M. Balls, *Comp. Biochem. Physiol.*, 1974, **47A**, 567.

Many aspects of the anabolism of glycogen from UDP-D-glucose, first postulated for liver extracts by Leloir and Cardini in 1957, are well established,[255, 256] and the suggestion that the synthesis of glycogen is initiated by a protein has received further support.[257, 258]

A micro-method for the diagnosis of type II (Pompe's) glycogen-storage disease is applicable to samples within 7—14 days after amniocentesis during the 14th—16th weeks of pregnancy.[259, 260] This method provides diagnosis more rapidly than previous methods. Further studies (using a bacterial isoamylase) on the liver glycogen from a male child with type IV glycogenosis showed that the pathological form of the polysaccharide does not have an amylopectin-like structure.[261] The liver-phosphorylase deficiency that occurs in type VI glycogenosis has been assessed by biochemical analysis of hepatic biopsy specimens.[262] A low-molecular-weight form of glycogen occurs in at least one form of glycogen-storage disease.[263]

Studies with foetal rat liver in culture suggested that the increase of glycogen occurring in late pregnancy requires the presence of glucocorticoids to induce glycogen synthetase and of insulin to activate the enzyme.[264]

Glycogen synthetase from yeast has been purified; its properties suggest that the phosphate group, whose presence determines that the enzyme is in the D-form, is close (*i.e.* within about 50 amino-acid units) to either the *N*- or the *C*-terminus of a polypeptide chain consisting of about 650 amino-acid residues.[265, 266] The effects of insulin on glycogen synthetase have been discussed.[267]

Rat-liver glycogen-synthetase D, whose activity is increased by the presence of D-glucose 6-phosphate, contains eight mercapto-groups per subunit; these groups partake in expression of the enzyme's activity and in its binding to glycogen to produce a complex.[268] Another form of the rat-liver enzyme (designated D′), which is absolutely dependent on D-glucose 6-phosphate for activity, has been prepared by incubating the D-form with magnesium chloride.[269] The physiological significance of this observation is unknown.

The mode of action of glycogen synthetase D (from human polymorphonuclear leucocytes) is believed to involve a rapid-equilibrium, random bi-bi mechanism in

[255] W. Stalmans and H. G. Hers, in 'The Enzymes', ed. P. D. Boyer, 3rd edn., Academic Press, London and New York, 1973, p. 309.
[256] A. J. Parodi, J. Mordoh, C. R. Krisman, and C. F. Leloir, in ref. 103, p. 409.
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[258] C. R. Krisman and R. Barengo, *European J. Biochem.*, 1975, **52**, 117.
[259] H. Galjaard, M. Mekes, J. E. De Josselin De Jong, and M. F. Niermeijer, *Clinica Chim. Acta*, 1973, **49**, 361.
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[265] K.-P. Huang and E. Cabib, *J. Biol. Chem.*, 1974, **249**, 3851.
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[268] M. J. Ernest and K.-H. Kim, *J. Biol. Chem.*, 1974, **249**, 5011.
[269] N. Abe and S. Tsuiki, *Biochim. Biophys. Acta*, 1973, **327**, 345.

which the attachment of D-glucose 6-phosphate is a prerequisite for interaction with substrate UDP-D-glucose.[270] Enzymic conversion *in vitro* of the D form into the I form of the enzyme was barely inhibited by ATP at low levels of glycogen, but it was markedly inhibited by ATP when the level of glycogen was increased; this may be of significance in controlling expression of the enzyme activity.[271] Other studies with rat-liver preparations have implicated the inhibitory effect of ATP on either synthetase phosphatase or synthetase D or, possibly, both.[272] A phosphoprotein phosphatase (isolated from bovine heart) catalysed the conversion of heart glycogen-synthetase D into the I form and also that of skeletal muscle phosphorylase *a* into the *b* form.[273] This finding supports other studies, which have demonstrated at least one mechanism whereby the synthesis and the catabolism of glycogen act co-operatively. Thus, administration of D-glucose to fed rats caused a rapid and an extensive inactivation of the liver phosphorylase; glycogen synthetase was activated only when the level of phosphorylase *a* dropped below a threshold value (equivalent to about 10% of the total phosphorylase level).[274]

The rates of enzyme transfer of sugars from UDP-3-deoxy-D-*ribo*-hexose, UDP-4-deoxy-D-*xylo*-hexose, and UDP-6-deoxy-D-glucose to glycogen have been compared with those from UDP-D-glucose and UDP-2-deoxy-D-*arabino*-hexose.[275] All compounds acted as substrates, but were less effective than was UDP-D-glucose.

The dephosphorylated (*b*) form of glycogen phosphorylase from pig kidney has been purified and shown to have a molecular weight of about 1.95×10^5; it requires AMP for expression of its activity.[276]

The purification and amino-acid compositions of isoenzymic forms of pig glycogen phosphorylase have been described.[277]

Explants of rat liver in culture have been used to study the effects of hormones on the induction of enzymes. This technique showed that an adrenergic receptor of the β-type mediated the effect of epinephrine on the metabolism of foetal rat-liver glycogen.[278]

The level of renal cortical glycogen in diabetes mellitus has been related to the degree of hyperglycaemia,[279] and there is extensive accumulation of glycogen in the jejunal mucosa of diabetic rats.[280]

Hypoglycaemia induced by injection of insulin was most pronounced in mice during early morning and least so at mid-day.[281] Moreover, the level of diaphragm-muscle glycogen during early morning was lower than that at mid-day. Hence, the results obtained following a single dose of insulin at an arbitrary time are likely to be misleading.

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A marked increase in the urinary excretion of bound 2-amino-2-deoxy-D-glucose has been noted in experimental diabetic animals.[282] The decreased response to insulin shown by rats injected daily with 2-amino-2-deoxy-D-glucose needs further investigation, in order that the relation between glycogen and glycoprotein syntheses can be compared.[283]

Red cells are normally deficient in glycogen stores, since enzymes in the degradative pathway are more active than those on the anabolic route.[284] Glycogen accumulates in the red cells of patients with a deficiency of either amylo-1,6-glucosidase or phosphorylase.[285]

Glycogen has been shown to be used more extensively by the muscles of chronic alcoholics than by those of controls during exercise.[286]

In contrast to earlier belief, the epithelium lining a distal segment of the tubuli recti and neighbouring parts of the rete testis in the guinea-pig has been shown to store large amounts of glycogen.[287] Non-germinal cells of rat testis, which normally contain little, if any, glycogen, stored the polysaccharide when the organ was subjected to γ-irradiation.[288]

The level of glycogen in the uterus of rats has been shown to increase progressively during pregnancy, whereas that in the liver has been shown to decline.[289] Repeated injections of oestrogen into rats led to increased amounts of glycogen in the uterus following the first injection, but no increases were noted thereafter.[290] The reason for this is not clear.

Allatectomy in the female house fly (*Musca domestica* L.) led to reduced levels of glycogen in the body; the levels of both glycogen synthetase and phosphorylase were reduced, but not that of trehalose synthetase.[291]

Glycosaminoglycans

A great deal is known about the structures and the biosynthesis of connective-tissue proteoglycans, and these areas have been reviewed.[292, 293] The effects of alkali on these macromolecules are of considerable interest, and the β-eliminative fragmentations of uronic-acid-containing polymers have been discussed in detail.[294]

The function of glycosaminoglycans during development is an exciting one. The mechanism of cytodifferentiation of cartilage cells during embryonic growth is but poorly understood, although several aspects of proteoglycan synthesis

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have been clarified.[295] The heterogeneity of proteochondroitin sulphates produced by various regions of chick epiphysis, such as the tibia and the femur, appeared to be a function of the state of cell differentiation.[296]

Proteoglycans from tissues can be extracted with 4M-guanidinium chloride, followed by 7M-urea; chromatography on DEAE-cellulose can be effected with the latter solution, affording a useful procedure for tissues containing only small amounts of proteoglycan.[297]

The predominant glycosaminoglycan in rabbit serum (100 μg per 100 ml) and in rabbit marrow cells (150 μg per 100 mg dry weight) has been identified as chondroitin 4-sulphate.[298] Undersulphated chondroitin 4-sulphate is the predominant form of glycosaminoglycan in human serum, which contains a total of 168—272 μg per 100 ml.[299]

The role of hyaluronic acid in joint lubrication has been extensively discussed.[300] Studies on the immunology of cartilage proteoglycan have suggested that the 'link-glycoprotein' is species-specific.[301, 302]

Glycoproteins rich in sialic acid and glycosaminoglycans present in chromaffin granules of adrenal medulla might be involved in the storage and release of biogenic amines, but this aspect requires further study.[303] Certainly, acetylcholine induced an increase in the secretion of sulphated glycosaminoglycans from perfused adrenal glands of cats.[304]

β-Aminopropiononitrile, which has long been used to produce experimental lathyrism, either is, or gives rise to, a potent metabolic poison; it also prevents the maturation of collagen. Its use led to a decreased rate of production of ATP and, by this means, reduced the rate of formation of glycosaminoglycans.[305]

The results of experiments with normal and polycythaemic mice suggested that increases in the levels of chondroitin sulphate and hyaluronic acid occur in the spleen when erythropoiesis is suppressed.[306] Glycosaminoglycans may thus play a role in erythropoiesis, as earlier proposed.[307] A system that allows investigation of the biosynthesis of glycosaminoglycans by corneal epithelium *in vitro* has been established.[308, 309] The observation that physiological concentrations of Ca^{2+} ions stimulated the uptake of sulphate into cartilage *in vitro* is of considerable interest.[310]

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The half-life of glycosaminoglycans in human articular cartilage is probably about 200—300 days, which is much longer than originally believed.[311] 2-Acetamido-2-deoxy-D-galactose 4,6-disulphate has been identified in rat urine, but its turnover in the animal is rapid.[312] It is, therefore, unlikely to arise by turnover of the glycosaminoglycan pool.

The distribution of proteoglycans in guinea-pig epiphyseal cartilage has been investigated by electron microscopy after the tissue had been stained with Ruthenium Red or Alcian Blue.[313] The healing of wounds is accompanied by an increased synthesis of sulphated glycosaminoglycans within a zone limited to *ca.* 5 mm from the edge of the wound.[314]

The properties of various glycosaminoglycans in mouse embryos (prior to chondrification), yolk sacs, trophoblasts, and deciduas differ with time.[315] Changes in the compositions and structures of human placental glycosaminoglycans during development have been measured.[316] The pattern of synthesis of glycosaminoglycans during the critical early stages of development of embryos of the frog *Rana pipiens* suggested that the molecules are involved in a number of processes.[317] There is also an increase in the amounts of glycosaminoglycans, of which at least 60% is hyaluronic acid, in the secondary palates of rats during the critical stages of palatogenesis, but the biological importance of this finding has not been defined.[318] Mn^{2+} ions appear to be important to the synthesis of glycosaminoglycans in the developing inner ear, and this observation may be of more general significance.[319]

The production of sugar-containing macromolecules by cells in culture is a problem of paramount importance, and many aspects thereof are discussed below. Cultures of chondrocytes and of limb bud cells were stimulated to produce large amounts of free chondroitin sulphate by β-D-xylopyranosides. Other cell lines (*e.g.* those of rat glial cells, mouse neuroblastoma, and rat hepatoma) that normally synthesize only small quantities of sulphated glycosaminoglycans can also be stimulated in this way.[320] The relative amounts of the various acidic glycosaminoglycans produced by cells in culture differed according to the amount of 2-amino-2-deoxy-D-glucose in the media, indicating the need for care in interpreting the results of studies conducted with fibroblasts in culture.[321] This finding may be related to the existence of topographically different pools of intermediate, hexosamine-containing precursors for the glycosaminoglycans, as there appear to be for hyaluronate and heparan sulphate in bovine arterial cells.[322] The metabolic relation of different intracellular pools of chondroitin

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sulphate proteins in the cartilage of calf ribs in culture has been examined.[323] Colchicine and vinblastine, which interfere with microtubule-mediated cellular functions, inhibited the synthesis of glycosaminoglycans by chondrocytes in culture.[324]

Chondrocytes have been shown to contain, probably in the lysosomes, an *endo*-polysaccharidase and a sulphatase which appear to be involved in intracellular catabolism of the chondroitin sulphates.[325] Variations in the levels of these enzymes, possibly due to changes in the lysosomal membranes, may underlie alterations in the nature of the glycosaminoglycans with age. The proportion of chondroitin 6-sulphate relative to chondroitin 4-sulphate in normal human urine increases with age in adults.[326, 327] The water content of gels prepared from glycosaminoglycans isolated from intervertebral discs of older horses and cattle was lower than that in younger animals.[328] This has been attributed to decreased entropic interactions of the proteoglycan with the gelatin matrix.

Histochemical methods have revealed that the Malpighian tubules of the larval housefly (*Musca domestica*) contain a non-sulphated polysaccharide which is not glycogen.[329] Separation and more extensive characterization of this polysaccharide are desirable.

The separation and quantitation of glycosaminoglycans in tissues and body fluids are as important as ever. Acidic glycosaminoglycans can be separated by thin-layer chromatography on cellulose using either ethanolic solutions of calcium chloride[330] or mixtures of formic acid, ammonia, methanol, and H_4edta.[331] A modification of an earlier procedure[332] has allowed good separations of glycosaminoglycans by two-dimensional electrophoresis on membranes of cellulose acetate.[333, 334] The levels of keratan sulphate in urine can be assessed by the latter technique,[334] and also by t.l.c. on silica gel.[335] Differentiation of urinary glycosaminoglycans by electrophoresis is useful in the diagnosis of mucopolysaccharidoses, for which routine screening methods are available.[336]

The incorporation of sulphate into pig costal cartilage *in vitro* was stimulated by somatomedin.[337] Glycosaminoglycans can be determined by a method that involves the interaction of scandium complexes thereof with Xylenol Orange; hafnium complexes of glycosaminoglycans can also be used.[338] Assays for 100 μg of glycosaminoglycans are accurate, with no interference from albumin, RNA, or mucin.

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Hyaluronic acid has been measured by a capillary turbidity procedure, which can be adapted for assays of the hyaluronidase activities of solutions.[339] The sugars present in urinary glycosaminoglycans can be assayed by g.l.c., if the polymers are reduced with sodium borohydride and then methanolysed; the patterns obtained on gas chromatograms can be used diagnostically for the mucopolysaccharidoses.[340]

Nephelometry has been used in the determination of glycosaminoglycans eluted from chromatographic columns.[341] While the technique is more sensitive than colorimetric or turbidimetric methods, it is also less specific. The polydisperity of chondroitin 4-sulphates isolated from bovine nasal septa and from lamprey cartilage has been shown (polyacrylamide gel electrophoresis) to be due to a continuum of varying chain length.[342]

L-Idopyranosyluronic acid residues in dermatan sulphate were found to be oxidized more rapidly by periodate ions than D-glucopyranosyluronic acid residues in other molecules; these results could indicate the $^4C_1$ conformation for L-idopyranosyluronic acid residues, so that O-2 and O-3 are equatorially disposed.[343] *X*-Ray diffraction studies have shown that L-idopyranosyluronic acid residues in crystalline dermatan sulphate have the $^4C_1$ conformation, even though the molecule may have an eight-, a three-, or a two-fold helix.[344] Conditions developed for the Smith degradation of dermatan sulphate yielded, *inter alia*, a product tentatively identified as L-threonic acid.[345] Some of the L-iduronic-acid-containing residues in pig-skin dermatan sulphate resisted the action of chondroitinase ABC, and this may be due to the presence of unsulphated 2-acetamido-2-deoxy-D-galactosyl residues.[346]

Not surprisingly, the interaction of heparin and chromatin *in vitro* led to extensive structural modifications of the protein.[347] Heparin bound serum proteins to form complexes, both *in vitro* and *in vivo*, at concentrations used therapeutically, although the nature of all the proteins involved is not known.[348] Heparin was shown to be a potent inhibitor of initiation of the translation of rabbit globin mRNA,[349] and it also acts, at least *in vitro*, as an allosteric modifier of post-heparin lipase.[350]

Heparin has been demonstrated to occur in two molecular-chain conformations, corresponding to two distinct crystal lattices, whose formation depends on the relative humidity.[351]

The biosynthesis of heparin involves the conversion of many of the D-glucopyranosyluronic acid residues into L-idopyranosyluronic acid residues within the polymer. There is good evidence that this epimerization and the sulphation step

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are interlinked.[352] This hypothesis is supported by the finding of increased amounts of both L-iduronic acid and ester sulphate in various fractions of heparin from pig intestinal mucosa.[353]

Heparin sulphamidase present in the lysosomes of rat spleen has been shown to be distinct from the arylsulphatases.[354]

Heparan sulphates A—D from beef-lung tissue differed with respect to their electrophoretic mobilities, optical rotations, and molecular weights, the distribution of *N*-acetyl and *N*- and *O*-sulphate residues, and in their behaviour with enzymes.[355] Heparan sulphate C was homogeneous with regard to its molecular weight.

Insulin has been reported to stimulate the production of hyaluronic acid by chick-embryo skin *in vitro*.[356] Bovine-brain hyaluronic acid appears to be devoid of arabinose. The presence of a carbohydrate–protein linkage could not be demonstrated in vitreous-humour hyaluronate,[357] but silicon may be involved in stabilizing the structure.[358]

Hyaluronic acid functions as an aggregating factor for proteoglycans in bovine nasal cartilage,[359] and it has been confirmed that a decasaccharide is the smallest effective inhibitor of this interaction.[360] The protein backbones (mol. wts. 4.5×10^4 and 9×10^4) of the proteoglycan retained the ability to bind to hyaluronic acid after being released from the aggregate.[361]

Treatment of hyaluronate with 8M-urea caused it to adopt a new conformation, which reverted to the original conformation under appropriate conditions.[362] *X*-Ray diffraction analysis has revealed that the two-fold helical conformation of chondroitin 4-sulphate persists following complexation with hyaluronic acid.[363] The proportion of proteoglycans linked to hyaluronic acid in extracts of pig laryngeal cartilage was shown to be greatest at pH 4.5.[364]

Slices of developing rooster comb have been used to study the formation of hyaluronic acid *in vitro*; the hyaluronic acid formed was identified by ion-exchange chromatography on Ecteola-cellulose and by enzymic degradation with ABC lyase.[365] The levels of hyaluronic acid synthetase rose during the growth phase of cultured mouse B-6 cells, but they were suppressed by the addition of 5-bromodeoxyuridine.[366]

The relationship between the isoenzyme profiles of staphylococcal hyaluronidases and staphylococcal diseases has been explored.[367]

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A keratan sulphate isolated from proteinase digests of bovine intervertebral discs or bovine nasal septa also contains residues of D-mannose, sialic acid, L-fucose, and 2-acetamido-2-deoxy-D-galactose in the ratio 1 : 3 : 1 : 1.[368] The sugars appear to be linked to the polypeptide backbone in two ways; one of the linkage types is labile to alkali. The alkali-labile linkage involves 2-acetamido-2-deoxy-D-galactose, which is substituted at O-3 by *N*-acetylneuraminyl-D-galactose.[369] Stretched films of a keratan sulphate from bovine cornea were found (*X*-ray diffraction) to contain the molecule in the form of a two-fold helix, with an axial rise of 0.945 nm per disaccharide residue.[370]

A high yield of a new disaccharide disulphate has been obtained from a chondroitin polysulphate isolated from king-crab cartilage; it was characterized as 2-acetamido-2-deoxy-3-*O*-[β-D-glucopyranosyluronic acid 2(3)-sulphate]-D-galactose 4-sulphate.[371] A polysaccharide from the test of the tunicate *Halocynthia roretzi* appears to be a polymer comprising 4-linked residues of 2-acetamido-2-deoxy-β-D-glucopyranose 6-sulphate.[372]

Density-gradient centrifugation of disassociative extracts of cartilage yielded a proteoglycan that was able to complex with lysozyme *in vitro*.[373] It is not known if this observation is indicative of the occurrence of a complex between the components *in vivo*, but the low levels of lysozyme in most cartilages suggest that complexation, even if it does occur, makes little contribution to maintaining the structural integrity of the tissue. The interaction of collagen with chondroitin 6-sulphate has been further investigated,[374] and chiroptical procedures have been used to examine the interactions of the chondroitin sulphates,[375] hyaluronic acid, heparan sulphate, and keratan sulphate [376] with cationic polypeptides. The ability of acidic glycosaminoglycans to interact with the surfaces of red cells is unexplained in functional terms.[377]

The chemical pathology of the glycosaminoglycans continues to be of interest. The biochemical bases of many of the inborn errors of mucopolysaccharide metabolism are well established.[378] A less well-defined type is characterized by the excretion of a chondroitin 6-sulphate of low sulphate content.[379] Methods for the enzymic diagnosis of a deficiency of glucuronidase in sera, plasma, urine, leucocytes, cultured fibroblasts from skin, and amniotic fluids have been described.[380]

Different low-molecular-weight oligosaccharides were shown to be present in the urine of patients with Sanfilippo A and B diseases.[381] The natures of a

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number of amino-sugar-containing acidic components of low molecular weight that are present in the urine of patients having several types of mucopolysaccharidosis have been determined,[382] and the patterns of urinary acid glycosaminoglycans in patients with Hurler, Morquio, and Scheie syndromes have been assessed.[383] The effects of hyaluronidase on the breakdown and excretion of glycosaminoglycans in Hurler and Hunter syndromes have also been examined.[384, 385]

It has been confirmed that cultured skin fibroblasts and liver homogenates of patients with Sanfilippo A disease are deficient in heparan sulphate *N*-sulphamidase activity.[386] Cultured amniotic cells also appeared to show a deficiency of this enzyme if the foetus is affected with Sanfilippo A disease.[387] The use of cultured amniotic cells for diagnostic purposes may be preferable to the assay of glycosaminoglycans in amniotic fluid, since false results have been obtained by the latter procedure in searches for Hunter and Hurler foetuses.[388] The levels of acid glycosidases in cultured fibroblasts from patients with Maroteaux–Lamy disease are lower than those from normal fibroblasts;[389] this is not the fundamental defect, which may arise from a deficiency of arylsulphatase B.[390]

Iliac crest cartilage of two children with Morquio's disease was found to contain above-average amounts of keratan sulphate and chondroitin 6-sulphate, but less than the normal amount of chondroitin 4-sulphate.[391] There appears to be an increased rate of synthesis of hyaluronic acid in cultured fibroblasts from patients with Marfan's disease.[392]

Patients with LeRoy's I-cell disease have elevated levels of acid phosphatase, acid phosphodiesterase, β-D-galactosidase, α-D-mannosidase, α- and β-D-glucuronidases, 2-acetamido-2-deoxy-β-D-glucosidase, and 2-acetamido-2-deoxy-β-D-galactosidase in their sera.[393] Cultured fibroblasts also have elevated levels of these enzymes. The results suggest that I-cell disease, unlike the other mucopolysaccharidoses, is due to an increased leakage of enzymes from the lysosomes.

The possibility of enzyme therapy for patients with mucopolysaccharidoses has continued to be explored. 2-Acetamido-2-deoxy-α-D-glucosidase supplied to cultured fibroblasts of Sanfilippo B patients was taken up by pinocytosis, and the enzyme became localized in the lysosomes.[394]

Other aspects of the chemical pathology of glycosaminoglycans are assuming increasing importance. The agglutination of ascites hepatoma cells by Ruthenium

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Red occurs by a mechanism which is inhibited by acidic glycosaminoglycans, but not by haptenic inhibitors of plant agglutinins.[395] Other cells (*e.g.* Rous ascites sarcoma and HeLa cells) that bind Ruthenium Red are only weakly agglutinated. The distribution of the types of acidic glycosaminoglycans present in ascites hepatoma cells differs, in part, from that in liver cells and in liver-cell plasma membranes.[396] Some of the differences may possibly be induced by growth in the ascites fluid. Combined administration of cyclophosphamide and vitamin A to ascites-tumour-bearing rats led to a decrease in the amount of tumour-associated glycosaminoglycans.[397]

It appears that *in vivo* synthesis of sulphated proteoglycans and their retention thereafter in the matrix are modulated by cell and serum factors; this deduction was based on experiments conducted with cultured human chondrocytes.[398] Chondrocytes from osteoarthritic patients deposited glycosaminoglycans into the cell layers more slowly than did those from controls.

2-Deoxy-2-propionamido-D-glucose had an inhibitory effect on the synthesis of glycosaminoglycans by human joint capsule and by synovial tissue in culture.[399] The use of this compound could be important in studies of the inflammatory response of tissues.

Examination of synovial fluids has been used in the diagnosis of amyloidosis,[400] and the urinary excretion of glycosaminoglycans by patients with collagen diseases has also been examined.[401] An increased urinary excretion of acidic glycosaminoglycans was noted in patients with Weber-Christian disease, but the significance of this is not known.[402] The glycosaminoglycan content of nucleus pulposa was found to be lower, and the collagen content to be higher, than normal in patients with idiopathic scoliosis.[403]

Bone, Cell, and Tissue Glycoproteins

The catabolic route for many, but probably not all, glycoproteins may involve the action of an *endo*-2-acetamido-2-deoxy-D-glucosidase, which, *in vitro*, was able to cleave (1 → 4)-linked 2-acetamido-2-deoxy-D-glucosyl residues occurring in egg-albumin glycopeptides.[404] This enzyme is present in liver, kidney, and spleen of rats and pigs. It did not cleave glycopeptides from IgG and calf thyroglobulin, so that the full implications of these observations are not clear.

The levels of UDP-galactose:glycoprotein galactosyltransferase of rat tissues have been shown to be high in foetal liver, lung, and brain, but they decrease during gestation; the enzyme in embryonic liver appears to be closely similar to, if not identical with, that in adult livers.[405] The reason for the presence of large amounts of carbohydrate in the glycoproteins of human lung tumours is not

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known, but the change results from metabolic abnormalities in the cancer cells.[406]

The ability of the dye carbocyanine to differentiate between nucleic acids and proteins and between glycoproteins and sulphated glycoproteins is likely to assist in pathological diagnoses.[407]

A glycoprotein (mol. wt. 5×10^4) isolated from bovine cortical bone was found to contain sialic acid (4%), hexosamine (3%), and neutral sugars (14%); small amounts (20 mg per 100 ml) of the glycoprotein are also present in bovine sera.[408]

Intravenous administration of 2-amino-2-deoxy-D-galactose to rats led to decreased rates of secretion of proteins and glycoproteins by the liver.[409] The incorporation of uridine and guanosine into liver slices *in vitro* was reduced.[410] A mannosyl-transferase in liver mitochondria catalysed the transfer of D-mannose from GDP-D-mannose to an endogenous glycoprotein acceptor;[411, 412] the enzyme is localized in the inner membrane.[413] The demonstration that D-galactosyltransferase isolated from rat-liver Golgi bodies is inhibited *in vitro* by puromycin indicates the need for special care in interpreting the results of experiments concerned with the biosynthesis of glycoproteins and glycolipids in which this antibiotic is used.[414] Lysolecithin was found to stimulate markedly a membrane-bound glycoprotein:galactosyltransferase, and it may have a regulatory function.[415]

The level of sialyltransferase in rat ascites hepatoma cells has been reported to be lower than that in normal rat-liver homogenates; the significance of this finding is not obvious, particularly as it is not known whether or not the enzyme is subject to metabolic regulation.[416] Injection of inactivated influenza vaccine into mice reduced the levels of hepatic microsomal glycosyltransferases (as assessed *in vitro*),[417] but the levels were enhanced in myxovirus-infected animals.[418] Isoenzymic forms of glycosyltransferases from this and from other organs can be separated by isolectric focusing.[419]

D-Mannosyl-, D-galactosyl-, 2-acetamido-2-deoxy-D-glucosyl-, 2-acetamido-2-deoxy-D-galactosyl-, and sialyl-transferase activities have been found in isolated hepatocyte nuclei; the fractions were checked for purity by electron microscopy and by the use of marker enzymes.[420]

Sialic-acid-containing glycoproteins are present in liver microsomal preparations as constitutive membrane components,[421] and a β-D-mannosyl linkage is

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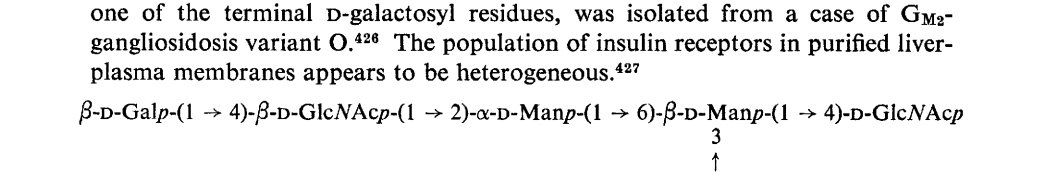
present in one or more of the glycoproteins occurring in microsomal membranes of rat liver.[422] A structure of the latter type is perhaps not unexpected. Microsomal fractions from the liver of the conger eel (*Conger vulgaris*) have been shown to contain glycosyltransferases which are able to transfer sugars from UDP-D-galactose, GDP-D-mannose, and UDP-2-acetamido-2-deoxy-D-glucose to endogenous protein acceptors.[423] Another teleost, *Labrus bergylta*, also contains these transferase activites in the hepatocytes.[424]

One of the glycopeptides that accumulates in the liver in type 1 G_{M1}-gangliosidosis has been identified as (26).[425] A closely similar oligosaccharide, containing an additional, non-reducing 2-acetamido-2-deoxy-D-glucosyl residue but lacking one of the terminal D-galactosyl residues, was isolated from a case of G_{M2}-gangliosidosis variant O.[426] The population of insulin receptors in purified liver-plasma membranes appears to be heterogeneous.[427]



(26)

A glycoprotein which specifically binds asialoglycoproteins has been isolated from rabbit liver.[428] The presence of intact, terminal sialic acid residues and Ca^{2+} ions is required for this glycoprotein to exhibit its binding specificity. Rat-liver Golgi bodies also contain a protein (or proteins) capable of binding asialofetuin.[429] A glycoprotein phosphodiesterase (mol. wt. 1.3×10^5) isolated from mouse-liver plasma membranes also possesses nucleotide pyrophosphatase activity, but it is distinct from the membrane 5′-nucleotidase.[430]

Mitochondrial glycosyltransferases from rat liver have been used to catalyse the transfer of D-glucose to collagen and of D-mannose to a modified immunoglobulin acceptor.[431] Some of the properties of these enzymes differed from those of glycosyltransferases in other parts of the cell.

Both simple and complex carbohydrate moieties are found in glycoproteins present in submitochondrial fractions of rat liver.[432]

It was reported earlier[433] that human hepatic hexosaminidase A could be converted into a form behaving like hexosaminidase B by the action of *Vibrio cholerae*. This change does not appear to be an enzymic conversion, but is catalysed by merthiolate, a preservative added to culture filtrates of *Vibrio*

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cholerae.[434] If this is so, the relation between the two forms of enzyme requires re-examination. A specific protein (mol. wt. 2×10^4) is present in homogenates of human liver; it cross-reacted with antibodies raised against 2-acetamido-2-deoxyhexosidases A and B, and is possibly a subunit of these enzymes.[435]

A mannosyltransferase activity present in calf pancreatic microsomes transferred D-mannose from GDP-D-mannose to a lipid acceptor to furnish a product very similar to α-D-mannopyranosyldolichol phosphate, which may be an intermediate in the synthesis of glycoproteins.[436]

A galactosyltransferase present in aorta intimal cells was found only in the soluble cytoplasmic phase; the enzyme was inhibited by CDP and UDP.[437] Glycoproteins linked to elastin through disulphide bridges in elephant aorta were shown to be rich in hexoses and to cross-react with antibodies raised against human aortic glycoproteins, but not with those raised against calf corneal glycoproteins.[438] These results imply similarities in the glycoproteins associated with elastin in related tissues.

The sugar sequence Neu*N*Ac*p* → D-Gal*p* → D-Glc*N*Ac*p*, which occurs frequently at the periphery of carbohydrate moieties of glycoproteins, appears to be present in a glycoprotein isolated from bovine aorta.[439]

Treatment of isolated rat adrenal cells with neuraminidase increased their response to cholera enterotoxin, and the production of both corticosterone and adenosine 3′,5′-cyclic phosphate by the cells was increased.[440] The results suggested that membrane-bound sialic acid residues may be involved in stimulation of adenylcyclase by the adrenocorticotrophic hormone.

The levels of renal cerebroside β-D-galactosidase and *p*-nitrophenyl β-D-galactosidase in sexually mature mice are about one-third higher in males than in females.[441] The effects of these differences on the catabolism of glycoproteins need to be elucidated. Glycoprotein:glycosyltransferase activities in rat kidney were raised following subcutaneous injection of animals with folic acid,[442] a technique that provides a model for studies of renal response to acute injury.[443] Higher levels of glycoprotein:galactosyltransferase activity were noted in kidney glomeruli following treatment with neuraminidase or mild acid or lysolethicin.[444] The results are of interest in connection with glomerular pathology.

No increase in the levels of hydroxylysine or disaccharides linked thereto was found in the basement-membrane collagen of diabetics.[445]

Glomerular basement membrane of human kidneys contains non-collagenous glycoproteins, which function at the membrane level.[446] There are also several

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glycoproteins, including one resembling collagen, in bovine basement membrane, but none contain 2-acetamido-2-deoxy-D-galactose.[447] Isolated kidney glomeruli can be made to synthesize glycoproteins, including those normally found in the basement membrane.[448]

A number of kidney lysosomal enzymes are glycoproteins wherein the carbohydrate moieties appear to protect the enzymes from attack by lysosomal cathepsins.[449] The synthesis and turnover of renal lysosomal glycoproteins involve the packaging of highly acidic, sialic-acid-rich macromolecules, which become less acidic with time owing to the loss of sialic acid residues.[450]

The particulate aminopeptidase isolated from pig kidney has been shown to contain about 25% carbohydrate comprised of D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and sialic acid; the enzymic activity was unaltered when all of the sialic acid residues, 60% of the neutral sugar residues, and 45% of the 2-acetamido-2-deoxy-D-glucosyl residues were removed enzymically.[451]

Rabbit-kidney alkaline phosphatase has been resolved into three isoenzymes by chromatography on DEAE-cellulose; only one of them underwent a change in electrophoretic mobility after treatment with *Clostridium perfringens* neuraminidase.[452] Detailed investigation of these differences was not attempted.

A calcium-binding protein (mol. wt. 2.7×10^4) isolated from necropsy specimens of human kidney is possibly a glycoprotein.[453]

The glycoprotein:sialyltransferases of calf and rat brains are very similar; isoenzymes in brain are probably located in membranes other than those of the synaptic complex, and also occur in neurons and in glia cells.[454] A mannosyltransferase that is probably involved in the biosynthesis of glycoproteins in the mitochondria of rat brain has been shown to differ from that present in microsomal portions of the organ.[455, 456]

Studies of the incorporation of radiolabelled leucine and L-fucose have revealed the presence of two glycoproteins (mol. wts. 9.9×10^4 and 9.6×10^4) in rat-brain plasma membranes.[457] The carbohydrate moieties of these glycoproteins are of two types, *viz.* those containing D-mannose and 2-acetamido-2-deoxy-D-glucose, and those also containing L-fucose, D-galactose, sialic acid, and 2-acetamido-2-deoxy-D-galactose.[458] Studies of the rate of metabolism of sialoglycoprotein GP-350 in adult rat brain showed that it has a relatively rapid turnover by comparison with that of other brain glycoproteins.[459] GP-350

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glycoprotein was found in large areas of calf brain and appeared to be associated with the nerve endings.[460]

The O-variant form of G_{M_2}-gangliosidosis (in which both hexosaminidase A and B activities are absent) leads to an accumulation of glycopeptides of relatively large molecular weight in the brain, but the levels of glycosaminoglycans are unaffected.[461] Hexosaminidase from human brain has been shown to be electrophoretically and immunologically distinct from the A- and B-forms of enzyme;[462] it has a molecular weight in excess of 2.0×10^5.[463]

Endogenous, membrane-bound neuraminidase was able to release sialic acid *in vitro* from glycoproteins and glycolipids present in homogenates of calf brain.[464]

A non-enzymic incorporation of 2-amino-2-deoxy-D-glucose into rat-brain synaptosomes and endoplasmic reticulum appeared to take place *in vitro* under certain experimental conditions, but the sugar was not necessarily bound covalently.[465] These experiments again point to the need for care in interpreting the results of studies relying on the incorporation of sugars.

Hexosaminidases A and B from human placenta are each composed of four subunits of molecular weight 3.3×10^4; it was possible to convert the A-form into the B-form by heating under carefully controlled conditions.[466] Human placental alkaline phosphatase contains D-mannose (3 residues), D-galactose (4 residues), L-fucose (0.6 residue), and D-glucose (0.8 residue per 7×10^5 g), in addition to sialic acid and hexosamine.[467]

Glycoproteins and glycolipids present in oxyntic cell microsomes of bullfrog gastric mucosa have been investigated.[468]

Sheep pancreatic ribonuclease [469] has been shown to occur in a non-glycosylated form (ribonuclease A) and other forms (B and C) that are glycosylated at the asparagine-34 residues.[470] Horse pancreatic ribonuclease is also a glycoprotein.[471] Bovine pancreatic deoxyribonuclease D differs from the C-form; the D-form has residues of D-galactose and sialic acid attached to it, whereas the C-form does not.[472] The polypeptide chains of the C- and D-forms are identical and differ slightly from those present in pancreatic deoxyribonucleases A and B. Glycopeptides prepared from pig pancreatic lipases L_A and L_B contain the amino-acid sequence Thr-Asn-Gly-Thr-Ile-Glu-Arg; the asparaginyl residue is probably glycosylated. Lipase L_A contains sialic acid residues in addition to residues of L-fucose, D-galactose, D-mannose, and 2-amino-2-deoxy-D-glucose, whereas lipase L_B contains the last four sugars only.[473] Golgi-rich fractions prepared

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from homogenates of adult rat pancreas contained 50% of the total UDP-galactose:glycoprotein galactosyltransferase activity.[474]

The affinities of the receptor sites for human chorionic gonadotrophin on cell membranes isolated from bovine corpus luteum were largely unaltered after the membranes had been treated with neuraminidase.[475, 476] Incubation of the membranes with either proteolytic enzymes or phospholipase A or C resulted in losses of binding ability. The chemical nature of the receptors for this glycoproteinaceous hormone has not been established. The binding sites for insulin on isolated cells from mouse mammary tissue were destroyed when the cells were treated with trypsin.[477] Binding sites specific for insulin are also present in cells from virgin mice, but binding of the hormone did not initiate a metabolic response.

Epidermal growth factor, which can be isolated from submaxillary glands of mice, stimulated the proliferation and keratinization of various epidermal tissues, both *in vivo* and *in vitro*.[478] This glycoprotein (mol. wt. 2.93×10^4), containing 2-amino-2-deoxy-D-glucose (1.7 residues) and 2-amino-2-deoxy-D-galactose (0.3 residue), is normally complexed with an arginine esterase of restricted specificity.

The receptor sites on hepatic membranes for asialoglycoproteins are well recognized. The affinities of substrates for the sites were shown to decrease in the order asialo-orosomucoid > asialothyroxine-binding globulin > asialofetuin > asialoceruloplasmin.[479] The finding that sera from patients with hepatocellular damage inhibited this binding could have important implications.

Studies of the levels of glycoprotein:glycosyltransferases during spermatogenesis in mice have indicated that active synthesis of glycoproteins occurs in testis germinal cells during, and immediately after, meiosis but probably not during the terminal stages of formation of the spermatozoa.[480] Deductions made from the results may require reconsideration.

Glycopeptides have been isolated following proteolysis of the isoenzymes of amylase from human parotid saliva.[481] There appears to be more than one site of attachment for the carbohydrate moieties in each of the isoenzymes, and cyst(e)ine is located close to one of the sites.

Rat parathyroid glands in culture appeared to synthesize glycoproteins containing 2-amino-2-deoxy-D-glucose, and to allow their passage into the medium.[482] The nature of the glycoproteins was not established. A neurospecific protein has been shown to contain carbohydrate, since it was stained by the periodic acid–Schiff reagent.[483]

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The predominant glycoproteins (mol. wts. 7.5×10^4 and 5.0×10^4) in frog sciatic ganglia were indicated to be transported rapidly, and the evidence for this has been summarized.[484] The Quaking mutation in mice has been shown to be associated with a severe deficiency of myelin in the central nervous system, and abnormal glycoproteins were found in the myelin.[485]

A number of properties of a major structural glycoprotein of sciatic nerve have been described.[486]

Animal Cells in Culture

As indicated above, carbohydrate moieties on plasma cell membranes are important for a number of reasons; they act as markers for the fluid regions of this organelle,[487, 488] and are also of interest in view of changes which they undergo at various stages of the cell's cycle.[489] The prosthetic groups may be involved in cell adhesion,[490] and their contribution to, or their effects on, membrane transformations in neoplasia are of great interest.[491] The mechanism of capping of lymphocytes, resulting from surface movements or from membrane flow, is not understood.[492] Cell structures regulating the mobility of various receptors appeared to be altered by binding to concanavalin A.[493]

A cultured lymphoblast line (SCRF 60_A), which is positive for θ-antigen but negative for immunoglobulin, carries a glycoprotein antigen of molecular weight 7.0×10^4; the antigen may be involved in neutralization of tumour viruses.[494]

It is worthwhile emphasizing that any modification (*e.g.* by injection or other treatments of a cell) of the plasma membrane may not be directly related to the change in biological activity observed. Many studies of glycoproteins of the plasma membranes of cells transformed by oncoviruses are reminiscent in approach to those made some years ago on the levels of glycoproteins in sera of cancer patients. A galactoprotein (mol. wt. 2×10^5) on the surface of BHK cells appears to be involved in controlling the initiation of DNA synthesis.[495] This was indicated from studies carried out on the cells with a temperature-sensitive mutant of a polyoma virus.

No differences were found in the levels of histocompatibility antigens on cultured human fibroblasts before and after infection with various mycoplasmas.[496] The antigen may function on the surface in a way that is essential for survival of the cell.

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It is often desirable to assay very low levels of sulphated mucopolysaccharides in cell cultures. This can be achieved by a technique that involves precipitation of ^{35}S-labelled materials on filter paper with cetylpyridinium chloride. Studies using this technique showed that the stimulatory effect of somatomedin on sulphated mucopolysaccharides produced in cultured cells is a general effect, rather than a specific effect on a particular type of polysaccharide.[497]

Thymocyte plasma membranes obtained (500 mg kg^{-1}) from calf thymus exhibited a 33-fold increase in 5′-nucleotidase activity.[498] The membranes contained sugars usually found in glycoproteins and carried binding sites for the agglutinins of kidney bean, lentil, mushroom, *Ricinus communis*, and wheat germ. A comparison of the glycoproteins with those from cultured lymphocytes is needed.

T-Lymphocyte cultures from dogs differed in their responses to phytohaemagglutinin according to the age of the animal from which the cells were obtained.[499] The best response was achieved with cells obtained from dogs between six weeks and six months old. This finding needs further biochemical development. Long-term, cultured lymphoid lines were demonstrated to contain up to six isoenzymes possessing α-L-fucosidase activity, but none of the isoenzymes was detected in the cells of a patient with fucosidosis.[500]

Proteins and glycoproteins in the plasma cell membranes of uninfected chick-embryo fibroblasts have been examined by discontinuous gel electrophoresis.[501] One of the predominant cell-surface glycoproteins has a molecular weight of 2.2×10^5.[502] A larger proportion of glycosaminoglycans was shown to be present in the surface of late-phase chick-embryo cells than in that of early-phase cells.[503] The finding that early-phase cells are relatively richer in glycopeptides may have important biological implications.

Mitochondria obtained from chick-embryo fibroblasts infected with the Rous sarcoma virus have been found to be able to synthesize greater amounts of glycoproteins than do normal cultures.[504] Both soluble and microsomal-associated galactosyltransferases have been isolated from chick-embryo fibroblasts; both endogenous and exogenous glycoproteins acted as acceptors of the hexose.[505] The microsomes of cells infected with Arborvirus appeared to contain an activating factor for the enzyme.[506] The mannosyltransferase activity at the stage of viral replication is enhanced.

The release of either BALB/c mouse 3T3 or Simian virus-transformed fibroblasts from glass or plastic surfaces by the action of ethylenebis(oxyethylenenitrilo)tetra-acetic acid left carbohydrate-containing materials and proteins,

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which may be important in cell-to-substrate adhesion, bound to the surfaces. One component was identified as hyaluronic acid,[507] although other glycosaminoglycans are also produced by the cells.[508] Related adhesive molecules may partake in the interaction of baby hamster-kidney and mouse L cells with binding surfaces.[509] The Oppenheimer–Humphreys aggregating factors of mouse teratoma cells may also include hyaluronic acid.[510]

The altered growth behaviour of BALB/c 3T3 cells, following transformation with Simian virus 40, was accompanied by changes in the plasma-membrane glycoproteins and glycolipids.[511]

Compared with normal cells in culture, lower levels of glycosyltransferases were involved in the synthesis of glycoproteins and glycolipids in virus-transformed mouse fibroblasts.[512] The enzyme activity UDP-2-acetamido-2-deoxy-D-galactose:haematoside 2-acetamido-2-deoxy-D-galactosyltransferase was completely absent in BALB/c 3T3 cells infected with mouse sarcoma virus.[513]

Cultures of baby hamster-kidney cells (BHK 21/C13) produced a glycoprotein closely similar to the urinary Tamm–Horsfall glycoprotein.[7]

Differences have been noted in the sizes of glycopeptides isolated from plasma membranes and from subcellular fractions of baby hamster-kidney cells (BHK 21/C13) in virus-transformed and untransformed states; glycopeptides from the transformed cells were larger.[514] Ethidium bromide was found to simulate virus transformation in this respect.[515] Increased mobility and redistribution of the receptors for concanavalin A were demonstrated on the surface of BHK 21 cells following infection with Newcastle-disease virus.[516]

Secondary cultures of cells of golden Syrian hamster embryos transformed by polyoma virus had much lower (3—12%) than normal levels of UDP-2-acetamido-2-deoxy-D-galactose:haematoside 2-acetamido-2-deoxy-D-galactosyltransferase activity, and the ganglioside pattern was also more simple.[517]

A selected clone (no. 15B) of Chinese hamster-ovary cells has been shown to contain drastically reduced binding affinities for ricin, phytohaemagglutinin, and soybean lectin, whereas it binds concanavalin A more readily.[518] The plasma membranes of the cloned cells contain less sialic acid, 2-acetamido-2-deoxy-D-glucose, and D-galactose, but more D-mannose, than do those of the parent cells.

Human tumour KB cells grown in culture contain at least three glycoproteins (apparent mol. wts. 9.2×10^4, 7.2×10^4, and 6.2×10^4) in the plasma membrane.[519]

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Five proteins present in HeLa cell membranes may be iodinated; three of them contain carbohydrate.[520] Antibodies prepared against similar fragments released into the medium inhibit the growth of HeLa cells. An increased amount of carbohydrate, particularly sialic acid, was shown to be associated with the glycocalyx of HeLa cells (derived from epitheloid-type cells) grown in the presence of prednisolone.[521] This appears to be due to a reduced shedding of the carbohydrate, rather than to a reduced extent of synthesis. The growth rate of the cells was reduced by comparison with cells grown in the absence of glucocorticoids, and the two events may be connected. Prednisolone represses the formation of an enzyme that releases sialopeptides. The results support the view that there is a correlation between cell proliferation and the density of sialoglycoproteins at the surface membrane.[522]

Growth-dependent changes associated with the carbohydrate–peptide linkage region were found in cultures of human KL2 diploid cells, and there may be a relation between this finding and the density-dependent inhibition of cell growth.[523]

An extract prepared from membranes of mouse leukaemia cells (L-1210) contained, among others, four glycoproteins with molecular weights of 8.4×10^4, 6.3×10^4, 4.4×10^4, and 3.3×10^4.[524] Heterologous antisera prepared against these glycoproteins were cytotoxic to L-1210 cells. Various biological effects were exerted by interferon on this type of cell, several of which probably resulted from changes in the cell surface.[525] Thus, the expression of H-2 histocompatibility antigens and the binding of concanavalin A were enhanced in cells treated with interferon.

Mouse L-929 cells appear to contain two classes (mol. wts. 1×10^5 and 5×10^4) of glycoproteins as cell-surface components.[526]

There are differences between the production of hyaluronic acid by primary cultures of normal iris melanocytes and by both melanotic and amelanotic lines of clones of B-16 mouse melanoma.[527] Whereas normal cells produced relatively large amounts of hyaluronic acid, the tumour cells produced little or none.

Morphologically differentiated, cultured neuroblastoma cells have been reported to take up 2-amino-2-deoxy-D-glucose from the medium more rapidly than do normal cells, and the incorporation of this hexosamine into glycoproteins is also faster.[528] It was possible to grow cloned cell-lines from mouse neuroblastoma in suspension and monolayer cultures to induce morphological differentiation.[529] The major glycoprotein on the cell surface of the suspension cells has a molecular weight of 1.05×10^5, whereas that on the monolayer cells has one of 7.8×10^4.

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The chemical structure and biosynthesis of the glycoprotein coat of TA3 cancer cells have been investigated.[530]

The impaired catabolism of glycosaminoglycans in cultured fibroblasts from the skin of patients with Sandhoff's disease was normalized when 2-acetamido-2-deoxy-β-D-hexosidase A (from normal urine) was added to the culture media.[531] The B-isoenzyme was not taken up by the cells and was thus ineffective. The deficiency of the B-isoenzyme in this respect could result from the lack of a specific marker, probably a carbohydrate side-chain, required for pinocytosis.[532]

Important results have been obtained by the use of cell-fusion techniques. Fibroblasts from Tay–Sachs patients (lacking hexosaminidase A activity), when fused with Sandhoff fibroblasts (lacking both hexosaminidase A and B activities), yielded a culture containing heterokaryons that produced a hexosaminidase exhibiting the electrophoretic and stability characteristics of the A-enzyme present in normal fibroblasts.[533] By use of man–mouse somatic cell hybrids, it has been possible to show that a gene involved in expression of hexosaminidase A is linked to genes coding for mannosyl phosphate isomerase and pyruvate kinase-3.[534] It is likely that the gene needed for the hexosaminidase-A phenotype requires the gene for hexosaminidase B in order that expression of the A-gene can occur.

Hormonal Glycoproteins

Glycoprotein hormones are often measured by radioimmunoassay.[535] A critical step in such methods involves the separation of the free and antibody-bound tracers. Dextran-treated charcoal has been used for this purpose in an assay of luteinizing hormone, when the free tracer was adsorbed.[536, 537] Electrolytic procedures for the iodination of glycoprotein and protein hormones have often proved to be advantageous.[538]

The biological and physicochemical properties of a modified luteinizing hormone have been assessed.[539] The positions of the disulphide bridges in the α-chain of luteinizing hormone and of pituitary thyrotropin are from cystine residues at positions 11 and 14 to positions 35 and 36 (or 36 and 35), respectively, as well as linking positions 32 to 64, 63 to 91, and 86 to 88.[540] Carbohydrate moieties are located at positions 56 and 82.

Human chorionic gonadotrophin (mol. wt. 6.5×10^4) has been purified by isoelectric focusing, followed by gel filtration;[541, 542] a similar procedure has been

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applied to the desialylated hormone.[543] The electrophoretic heterogeneity of the glycoprotein hormone has been ascribed to differences in the degree of sialylation of individual molecules.[541] Both chorionic gonadotrophin and luteinizing hormone stimulated the conversion of ATP into adenosine 3′,5′-cyclic phosphate in mitochondria isolated from rat testes, but it is not known whether or not this effect operates on adenylate cyclase *in vivo*.[544]

Chorionic gonadotrophin was reported to have blood-group A activity (measured by haemagglutination inhibition), although it does not contain any 2-acetamido-2-deoxy-D-galactosyl residues.[545] If this finding is confirmed, it will be necessary to pursue the reason for the observed blood-group A activity with some urgency.

The amino-acid sequences proposed for the α- and β-subunits of human chorionic gonadotrophin show the presence of glycosylated asparagine-52 and -57 residues in the α-chain and two (13 and 30) in the β-chain, which also has glycosylated serine-121, -127, -132, and -138 residues.[546] The sequences at the four serine residues are Ser-Ser-Ser-Ser(X)-Lys-Ala-Pro, Pro-Pro-Pro-Ser(X)-Leu-Pro-Ser, Pro-Ser-Pro-Ser(X)-Arg-Leu-Pro, and Pro-Gly-Pro-Ser(X)-Asp-Thr-Pro, where Ser(X) represents the glycosylated serine residue. A number of the results differ from previous data.

Human follicle-stimulating hormone has been cleaved by treatment with urea, after which the α- and β-subunits were isolated by ion-exchange chromatography.[547] The amino-acid compositions of the subunits showed a number of differences from those reported earlier. Related procedures were applied to the equine hormone.[548] The amino-acid sequences of the α- and β-subunits of human follicle-stimulating hormone have been described in other reports.[549, 550] Each subunit carries carbohydrate moieties, and structures for these prosthetic groups have been proposed from the results of a number of studies.[551, 552]

The levels of follicle-stimulating hormone in anterior pituitary glands of control and heat-acclimatized male rats were the same per unit weight (about 9—10 I.U. per mg of gland), but the incorporation of lysine into proteins in the testes was higher in the second group.[553]

Information on the nature of glycoprotein–hormone receptors has become available. The glucagon-binding protein prepared from rat-liver plasma membrane has a subunit molecular weight of 1.9×10^5, and it also binds insulin weakly.[554]

Human follicle-stimulating hormone has been shown to be tissue-specific in its binding properties; with rat tissues as a model, the hormone binds extensively only to homogenates of seminiferous tubules.[555]

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Milk Glycoproteins

An acid glycoprotein from human colostrum has been shown to have a molecular weight of 3.1×10^4 and to comprise 68% carbohydrate; it probably contains at least seven *O*-glycosidically linked carbohydrate moieties.[556] The growth-promoting activity of this glycoprotein for *Lactobacillus bifidus* var. Penn has been assessed.[557] The carbohydrate moiety of sheep κ_A-casein has been located at one of the threonine residues in positions 135, 137, or 138, a region in which the sequence Ser-Thr-Pro-Thr-Thr-Glu occurs.[558] Whole casein from colostrum was found to be richer in κ-casein than normal casein, and it contains more carbohydrate and also 2-acetamido-2-deoxy-D-glucose, although an asparagine sequon is not present.[559]

A neuraminidase from influenza A2 virus cleaved sialic acid from *N*-acetyl-4-*O*-acetylneuraminyl-lactose [isolated from echidna (*Tachyglossus aculeatus*) milk] *in vitro*, whereas the bacterial neuraminidase from *Clostridium perfringens* was unable to utilize this substrate.[560]

Bovine, pig, and sheep colostrum each contain two forms of galactosyltransferase, one (mol. wt. 5.0×10^4) of which is unique to colostrum; the other form (mol. wt. 4.1×10^4) is probably derived by partial proteolysis.[561] Both enzymes act as *N*-acetyl-lactosamine synthetase and, in the presence of α-lactalbumin, as lactose synthetase; ovalbumin serves as a substrate for both enzymes *in vitro*. The kinetic properties of the galactosyltransferase from human milk have been studied.[562] The lactose synthetase activity of the A-protein from human milk was greatly decreased following treatment with reagents reactive towards thiol groups.[563] The reactive group on the protein, whether it is a thiol group or not,[564] is located in the neighbourhood of the binding site for UDP-D-galactose.[563] A massive rise in the rate of synthesis of lactose takes place during lactogenesis in rats, and it appears to result largely from increased levels of lactose synthetase A-protein and α-lactalbumin.[565] The possible significance of the complexes formed between α-lactalbumins and the A-protein in the presence of D-glucose, 2-acetamido-2-deoxy-D-glucose, and UDP-D-galactose has been discussed in relation to the regulation of lactose synthetase activity.[566]

Affinity chromatography can be used to obtain quantitative information on the interaction of an enzyme with a ligand; it has been used to determine the effect of 2-acetamido-2-deoxy-D-glucose and of D-glucose on the elution of the A-protein of human lactose synthetase from a column comprising α-lactalbumin coupled to agarose.[567]

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Another specific galactosyltransferase found in human milk catalyses the formation of 6-*O*-D-galactosyl-lactose from UDP-D-galactose and lactose.[568]

The glycoprotein lactoferrin may have an important function in inflammatory hyposideremia. On release from neutrophiles, iron-free lactoferrin removed iron from transferrin and was then selectively taken up by the reticuloendothelial system.[569] The precise structural and genetic relations between transferrin and lactoferrin have not been elucidated. Lactoferrin has been shown to be glycosylated at the asparagine residue in the sequence Asn-Gln-Thr-Gly.[570]

Serum Glycoproteins

A two-step procedure has been reported for the isolation of albumin, transferrin, fibrinogen, and two α-glycoproteins from rat sera, and the procedure should be invaluable in studies of the biosynthesis of α-glycoproteins.[571] The binding specificities of the progesterone-binding globulin, other than transcortin, in the guinea-pig have been studied.[572]

Useful procedures, involving either immunodiffusion [573, 574] or enzymes,[574] have been described for assay of the levels of α_1-antitrypsin in sera. α_1-Antitrypsin glycoprotein appears to be present at higher levels in the sera of patients with bronchial asthma than in controls.[575] Higher levels of this glycoprotein have also been reported in patients with rheumatoid arthritis.[576] Endogenous α_1-antitrypsin glycoprotein can also function *in vitro* as an inhibitor of human-leucocyte collagenases [577] and elastases,[578] human plasmin,[579] and the acid cathepsin of human alveolar macrophages.[580] It hydrolyses hepatocyte acid cathepsins.[581] The discovery of the way in which the carbohydrate moiety of this glycoprotein is involved in its various activities is awaited with interest. Unaltered α_1-antitrypsin from human plasma has a molecular weight of 5.2×10^4.[582] A study has been made of cirrhosis and of malignant hepatomas and their relation to deficiencies of α_1-antitrypsin,[583] and a comprehensive review has dealt with this polymorphic glycoprotein and deficiencies thereof.[584] A material (mol. wt. 1.8×10^4) isolated from normal liver cross-reacted with antibodies raised against α_1-antitrypsin, but differed electrophoretically from that isolated from a patient with an α_1-antitrypsin deficiency.[585]

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A surfactant protein from canine lungs has been found to contain a trypsin inhibitor which is electrophoretically indistinguishable from serum α_1-antitrypsin.[586]

Counter-immunoelectrophoresis is probably one of the most sensitive methods for detecting α-foetoprotein,[587] and radio-immunoelectrophoresis can also be employed for levels of 30—50 $\mu g\ ml^{-1}$.[588]

Units linked *O*-glycosidically to serine and threonine residues of fetuin have been shown to possess the structure Neu*NAc*p-(2 → 3)-β-D-Gal*p*-(1 → 3)[Neu*NAc*p-(2 → 6)]-D-Gal*NAc*p, but some of the units lack the (2 → 6)-linked *N*-acetylneuraminic acid residue.[589] α_2-Macroglobulin inhibited *in vitro* a specific collagenase from rabbit synovial cells.[590] Human α_2-macroglobulin can be fractionated by isoelectric focusing into four distinct pools of molecules, each containing different amounts of sialic acid and D-galactose.[591] Injection of α_2-macroglobulin into tumour-bearing rats inhibited growth of the tumour.[592] Very high levels of α_2-macroglobulin in the nephrotic syndrome were accompanied by lower-than-normal levels of Zn^{2+} ions.[593]

The level of α_2-glycoprotein–Zn in sera has been shown to rise from 2.5 mg 100 ml^{-1} in human foetus to about 9 mg 100 ml^{-1} for one-year-olds.[594] Each of the three carbohydrate moieties present in human ceruloplasmin appeared to be attached to asparagine sequons.[595] The dimensions and shape of ceruloplasmin have been determined from small-angle *X*-ray scattering.[596] The ceruloplasmins of neonates and adults appear to be identical,[597] although markedly increased levels have been noted in the sera of patients with active rheumatism.[598]

The diagnostic value of measurements of the levels of haptoglobin and of transferrin in sera has been evaluated.[599]

Comparison of the sequences of six cysteine-containing peptides derived from human lactoferrin with those adjacent to the cysteine residues in chicken ovotransferrin has revealed close similarities between the two, suggesting that lactoferrin and transferrin are derived from a common ancestral, iron-binding glycoprotein.[600] Human asialotransferrin was catabolized in rabbits much faster than was human transferrin, when each was injected separately.[601]

The first studies of the metabolism of the glycine-rich β-glycoprotein (a major component of the C3b-feedback pathway) indicated a very rapid turnover.[602]

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High-density apolipoprotein from the Rhesus monkey has been shown to contain carbohydrate (1.5%) consisting of 2-amino-2-deoxy-D-glucose (0.45%), D-galactose (0.22%), D-mannose (0.22%), L-fucose (0.07%), and sialic acid (0.52%).[603] The principal protein of human high-density lipoprotein was shown to possess a high helical content at neutral pH, but this decreased both in acidic and in alkaline media.[604] Neither apolipoprotein-Ala nor apolipoprotein-Gln(II) from man appears to contain an asparagine sequon for glycosylation.[605]

A threonine residue substituted by 2-acetamido-2-deoxy-D-galactose occurs in an apolipoprotein isolated from very-low-density human lipoproteins.[606] Its presence in the sequence Val-Arg-Pro-Thr(X)-Ser-Ala-Val suggests that the threonine sequon for 2-acetamido-2-deoxy-D-galactosylation contains a proline residue and either an arginine or a lysine residue,[607] but the evidence for this is not wholly convincing.

A specific glycoprotein has been found in sera and ascites fluids of mice bearing the Ha, but not the St, subline of Ta_3 mammary adenocarcinoma.[608] This glycoprotein, which arises from the plasma membrane of Ha cells, may block the immunoresponse to the Ha tumour.

A simple, reproducible procedure for the isolation of bovine prothrombin and of Stuart (X) factor has been described.[609] There is considerable homology in the amino-acid sequences at the *N*-termini of bovine prothrombin and Christmas (IX) and Stuart factors.[610] Immunological cross-specificity has been observed between antibodies to prothrombin and to Stuart factor, which have significantly different carbohydrate contents. Christmas factor has been purified from bovine plasma by a procedure involving chromatography on heparin–agarose; it was found to have a molecular weight of 5.55×10^4 and to contain hexose (10.6%), 2-acetamido-2-deoxyhexoses (6.5%), and sialic acid (8.7%).[611] Human Christmas factor can be isolated by isoelectric focusing; its isoelectric point ranges from 4.10 to 4.37.[612] The activated form of Stuart (Xa) factor from human serum has a molecular weight of 2.5×10^4 and contains carbohydrate (14%).[613]

The half-life for turnover of fibrinogen in dogs was found to be 38.5 ± 5.8 hours when a ^{125}I-labelled fibrinogen was used, but 119.4 ± 30.7 hours when a ^{75}Se-labelled glycoprotein was used.[614] The latter value is probably more nearly correct. The carbohydrate of human fibrinogen appears to be distributed on the β- and α-chains only, which probably carry two and one carbohydrate moieties,

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respectively.[615] A 2-acetamido-2-deoxygalactosyltransferase[616] in the sera of blood-group A and AB individuals had the ability *in vitro* to transfer the amino-sugar from UDP-2-acetamido-2-deoxy-D-galactose to glycoproteins and oligosaccharides possessing H activity.[617]

Immunoglobulins

Antibodies have considerable variation and adaptive expression,[618] and a number of such aspects pertaining to immunoglobulin E and other immunoglobulins have been reviewed.[619, 620] There is considerable interest in the nature of mouse myeloma proteins possessing binding specificities for such polysaccharides as dextrans[621, 622] and fructosans.[622] The binding site can accommodate a unit the size of a pentasaccharide.[621]

Quantitative analysis of immunoglobulins in sera can be achieved by chromotography on DEAE-cellulose.[623] The immunoglobulins of the South African clawed toad (*Xenopus laevis*) have been studied.[624]

The F_c-region of mouse immunoglobulin G2a contains the sequon known to be necessary for glycosylation of asparagine to occur.[625]

Plasma from a patient with IgG myeloma has been reported to have an increased capacity for binding calcium ions, but that from patients with cystic fibrosis is normal in this respect.[626] It is not clear how the carbohydrate moiety of IgG myeloma glycoprotein is involved.

A newly described plasma-cell dyscrasia was manifest biochemically as an IgGl serum protein with deletions in both heavy-chains and by the appearance of free F_c-fragments in urine and sera; chains with the deletion were present in urine also.[627] All the paraproteins are glycoproteins.

The heterogeneity of monoclonal immunoglobulin-G proteins revealed by isoelectric focusing requires further investigation.[628] It has been demonstrated that monoclonal immunoglobulins G with similar sugar contents possess different capacities to fix the complement, suggesting that sugars in the F_c-region play little or no part in this activity.[629] However, a rabbit IgG containing anti-pneumococcal activity lost its complement-fixing and opsonic activities if large parts of the sugar moiety were removed enzymically.[630]

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The subunit interaction of immunoglobulin G has been studied by calorimetry.[631] Mycobacterial glycopeptides, which contain arabinose and muramic acid and which bind to guinea-pig IgG2, were bound by both F_c- and F_{ab}-regions.[632]

High-resolution *X*-ray analysis of well-ordered crystals showed that human IgG has a $P3_121$ space-grouping and that the crystallographic diad relates the two halves of the molecule.[633]

Allotypic specificities of the heavy-chain of rabbit immunoglobulin G have been shown to be controlled by genes at a single locus, with three alleles a_1, a_2, and a_3.[634] The requisite sequon for glycosylation resulted from a_3 at positions 28—30 of the variable region [...Asn-Gly(Ala)-Ser...], whereas it has not been found in sequences so far reported for a_1 and a_2.

It will be of interest to know whether or not asparagine-75 in a number of α- and γ-chains is able to undergo 2-acetamido-2-deoxy-D-glucosylation, since there is a serine residue at the next-but-one position on the *C*-terminus.[635] The intermediate amino-acid residue is aspartic acid, which also may not permit glycosylation of asparagine-75; in any case, *X*-ray analysis (at 2 Å resolution) of the F_{ab}'-fragment of a myeloma protein (IgGl New) suggested that asparagine-75 may not be readily accessible.[636]

A heavy-chain disease involving an α1-immunoglobulin (Def) has been recognized; the protein has an internal deletion of almost all of the F_d-segment.[637] The position of the carbohydrate is not known. A myeloma glycoprotein from mice (MOPC 47A) also appears to have a deletion of about a hundred amino-acid residues.[638] The size of the binding sites of two IgA myeloma proteins for terminal, non-reducing β-D-galactopyranosyl residues corresponds approximately to that needed for a tetrasaccharide.[639]

Each unit of dimeric and tetrameric forms of human secretory IgA contains only one J-chain,[640] which appears to be linked through disulphide bridges to the penultimate cysteine residues of the α-chains.[641] J-Chain has been isolated also from mouse IgA myeloma protein.[642] Monomeric and polymeric forms of human IgA show different electrochemical properties, as demonstrated by polarography.[643]

Both F_{ab}- and $(F_c)_5$-fragments have been isolated from rabbit immunoglobulin M by digestion with papain in the absence of reducing agents.[644] The cyclic

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arrangement of the pentameric form of immunoglobulin M is believed to involve one J-chain per pentamer, arranged so as to bind to only two of the IgM molecules.[645]

New cases of heavy-chain disease involving μ-chains have been described. The paraprotein in one disease is devoid of light-chains,[646] whereas the J-chain is missing in another.[647] In neither case is the distribution of carbohydrate known.

The J-chains remained soluble when reduced and alkylated polymeric IgA and IgM were dialysed against non-dissociating, aqueous solvents, whereas the rest of the protein was rendered insoluble. This property forms the basis of an easy process for isolating J-chains.[648] The J-chain of pig IgM does not appear to be involved in aggregating the subunits,[649] although that of human IgM is associated with the F_c-region.[650] Some of the associative forces are due to linkages other than disulphide linkages.[651] The J-chain (mol. wt. 2.9×10^4) of human IgM appears to be a dimer, with the subunits (mol. wt. 1.5×10^4) tightly bound together by non-covalent forces.[652] Other smaller fragments have also been found.[653] J-Chain was reincorporated into the polymeric form of IgM if the immunoglobulin was reduced and then reoxidized, but it is not known whether the overall structure resulting is identical with the original.[654] The $(F_c)_{5\mu}$-fragment isolated from tryptic digests of human IgM has a molecular weight of 3.4×10^5.[655]

The levels of IgG, IgM, and IgA in the sera of patients with ankylosing spondylitis were found to be higher than those of controls.[656] The finding of IgD antiglobulins in some cases of juvenile rheumatoid arthritis is likely to facilitate further studies on this class of immunoglobulin.[657]

IgE has been found in hair from the heads of humans.[658] Immunocytomas involving the production of IgE will enable more definitive studies to be made of the carbohydrates in this class of immunoglobulin.[659, 660]

The ε-chains of a particular IgE myeloma protein each contain D-mannose (16), 2-acetamido-2-deoxy-D-glucose (12), D-galactose (7), sialic acid (5), and L-fucose (4 residues).[661] Another carbohydrate moiety, containing only D-mannose (6) and 2-acetamido-2-deoxy-D-glucose (2 residues), was shown to possess the 'core' structure (27) linked to an asparagine residue. It is unusual in having an α-linked

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2-acetamido-2-deoxy-D-glucosyl residue. Other glycopeptides have structures of the type more usually encountered.[662]

$$\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}N\text{Ac}p$$
(27)

Six antigenetically distinct classes of immunoglobulin (G_1, G_2, G_3, A_1, A_2, and M) have been identified in Indian buffalo serum.[663] Four of them (G_1, A_1, A_2, and M) were also found in whey colostrum.

A new subgroup of κ-type light-chains is now known as a result of sequence studies of a Bence-Jones protein.[664] The crystalline, monoclonal κ-chain (Rei) was found to lack the necessary sequon for glycosylation by 2-acetamido-2-deoxy-D-glucosyl residues.[665, 666] The sequence of another κ-type chain (Scw) has also been determined.[667, 668]

The total sequence of Bence-Jones protein Vor of the λ-type, which carries the Oz marker, has been determined.[669] Neither this protein nor the group IV (λ-type) protein Bau [670] possesses an asparagine sequon for glycosylation. A λ-type Bence-Jones protein (Ta) has been assigned to group IV by sequence determination at the *N*-terminus.[671]

The relation of the amyloid fibre protein (AS) in rheumatoid arthritis to the immunoglobulins is not clear, even though its amino-acid sequence has been elucidated.[672]

Blood Cellular Element Glycoproteins

The known differences between bone-marrow- and thymus-derived cells have been stressed in an important review of the structures, functions, and formation of lymphocytes.[673] The θ-alloantigen can be released from mouse T-cells by acid–urea treatment or by treatment with a non-ionic detergent (Nonident P-40), but neither procedure was effective with B-cells.[674] Separated T- and B-lymphocytes have been used as either stimulating or responding cells in mixed cultures.[675]

T-Lymphocytes can be blast-transformed by concanavalin A, whereas those of B-cells cannot. T-Lymphocytes from neonatal rats were preferentially agglutinated and blast-transformed by concanavalin A, whereas neonatal spleen lymphocytes were agglutinated by wheat-germ agglutinin but were not transformed by concanavalin A; spleen lymphocytes from adult rats were transformed

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[675] H. von Boehmer, *J. Immunol.*, 1974, **112**, 70.

by concanavalin A.[676] All the cells contain a galactosyltransferase on the surface. An interesting development has occurred in that spleen cells isolated from congenitally athymic (nude) mice did not undergo blastogenesis when subjected to oxidation with periodate ion under mild conditions.[677] These results indicate that only T-cells are stimulated by this mitogen.

The ontogeny of mouse-lymphocyte function has been examined by methods involving the use of mitogens.[678] The bases for the observed differences need to be explained with regard to glycoproteins and glycolipids at the cell surface.

The binding site for cholera toxin (mol. wt. 8.4×10^4) on the surface of lymphocytes involves carbohydrate residues.[679] However, the inhibitory action of cholera toxin on blastogenesis induced by concanavalin A cannot be attributed to blocking of the binding sites for the lectin.

The attachment of myxo- and paramyxo-viruses to T-lymphocytes probably proceeds by way of sialic acid residues, but at different sites.[680] B-Lymphocytes also have binding sites of a type not detected on the surface of T-lymphocytes.

Heterologous antisera to human lymphoid cells can be used to precipitate ^{125}I-labelled antigens present *in vitro* in lymphocyte membranes; labelling was achieved by means of the lactoperoxidase technique.[681] The glycoproteinaceous nature of antigens in the membranes of lymphocytes from thymus, tonsil, peripheral blood, and cultured cell-lines was assessed by this technique. The redistribution of lectin and other binding sites on mouse lymphocytes induced by hybrid antibodies probably results in a thermodynamically favoured aggregation of the surface components by altering the electrostatic-charge characteristics of the labelled molecules.[682] Thus, multivalent cross-linking is probably not required for patch reaction and for capping.

β_2-Microglobulin (mol. wt. 1.16×10^4) may be involved in the recognition units on human T-lymphocytes.[683] Thus, antiserum raised against β_2-microglobulin blocked the reactivity of lymphocytes towards allogeneic cells in mixed leucocyte cultures and also inhibited the transformation of lymphocytes induced by phytohaemagglutinin. However, little, if any, inhibition of the formation of T-lymphocyte rosettes was induced by sheep red-blood cells. The antiserum induced aggregation on the cell surface of β_2-microglobin, which capped together with the HL-A antigen; the cell was then resistant to the lytic action of antibodies to HL-A in the presence of complement.[684] These findings are explained by the fact (see also p. 319) that β_2-microglobulin constitutes a part of the HL-A antigen with which it associates at the cell surface.[685] β_2-Microglobulin has been shown (by immunofluorescence) to be present on membranes of polymorphonuclear

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leucocytes and on T- and B-lymphocytes, but it was not detected on erythrocytes.[686] HL-A antigen isolated from spleen contains β_2-microglobulin.[687, 688] Detergent-solubilized HL-A antigens have been purified by chromatography using an immobilized *Lens culinaris* lectin.[689] At least some of the electrophoretic heterogeneity of HL-A antigens isolated from cultured, human lymphoblastoid cells can be ascribed to differences in the contents of sialic acid.[690] Papain-solubilized H_2-A antigen has been shown to contain two subunits, comprising a glycoprotein (mol. wt. 3×10^4)[691] and β_2-microglobulin (mol. wt. 1.2×10^4).[691, 692] Several aspects of detergent- and papain-solubilized H_2-alloantigens from mice have been reviewed.[693]

HL-A antigens have been found in pre-Columbian mummies, a finding that may be of interest to anthropologists.[694]

The full significance of the relation between β_2-microglobulins and HL-A antigens to the transplantation of organs is unknown. The levels of β_2-microglobulin are considerably higher in human foetal sera than in maternal and adult sera.[695] Increased levels of β_2-microglobulin were also noted in the sera of a large proportion of patients with malignant tumours.[696] Certain features of the secondary and tertiary structures, as well as the primary sequence, of β_2-microglobulin are related to those of immunoglobulin.[697] However, the ability of a number of normal and malignant cells to synthesize β_2-microglobulin *in vitro* does not appear to be related to their ability to synthesize immunoglobulin.[698] This is clearly a fruitful area for further research.

Cell-surface immunoglobulin appears to be synthesized and transported in an analogous manner to secretory immunoglobulins. Cell-surface immunoglobulin has been tritium-labelled *in vitro* with appropriate derivatives of 2-amino-2-deoxy-D-glucose, D-galactose, and L-fucose; the results suggested that the order of incorporation of the sugars is the same as that in the secreted antibody.[699] However, it has been reported that IgM on cell surfaces contains only 2-amino-2-deoxy-D-glucose and D-mannose.[700] The predominant immunoglobulin (8*S* IgM) on the surface of mouse splenocytes was shown to be attached by way of μ-chains.[699] Stimulation of small, resting B-cells by a lipopolysaccharide mitogen

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from *Escherichia coli* induced changes in the carbohydrate composition of IgM.[701]

The membrane-associated immunoglobulin (IgM) from cultured Daudi cells (derived from a Burkitt lymphoma) has been shown to have a molecular weight of 2.65×10^5; the μ-chains have a molecular weight of 7.5×10^4 and the carbohydrate-containing K-chains have a molecular weight of 3.3×10^4.[702]

The specific α_2-neuraminoglycoprotein (C1 inactivator) on blast cells was found to be present in bone-marrow specimens of patients with blast leukaemia, and about 43% of the cells were coated; the glycoprotein was not detected in the cells of patients without neoplastic diseases.[703] Carcinoma cells were also shown to carry the glycoprotein to a greater extent than do cells from pleural and ascites fluids of non-neoplastic patients.[704] The diagnostic possibilities are obvious, and the findings could explain why tumour cells are often protected from the humoral immune system.

The major glycoprotein of horse-erythrocyte membranes has been shown to have a molecular weight of 1.13×10^5 by centrifugation in 5M-guanidinium chloride solution,[705] although a lower molecular weight (3.9×10^4) has been obtained by gel electrophoresis.[706] The major glycoprotein of human red-cell membranes has been confirmed to behave as a polymer of molecular weight 9.5×10^4 on gel electrophoresis.[707] The glycoprotein fraction isolated from red-cell ghosts of *Bos taurus* has been characterized.[708]

It is important to ask whether or not resealed cell ghosts have a topography identical with the erythrocytes from which they are derived. The labelling patterns of membrane proteins of cell ghosts and of erythrocytes obtained using a membrane-impermeable nitrene precursor [*N*-(4-azido-2-nitrophenyl)-2-aminoethyl sulphonate] suggested that differences do exist.[709] By contrast, the labelling of whole cells and cell ghosts by iodination with lactoperoxidase did not reveal any differences.[710] However, ^{125}I-labelling experiments with whole-cell ghosts and with resealed vesicle membranes indicated that a major and two minor glycoproteins present in human erythrocytes are able to penetrate the membrane.[710, 711] In other experiments, labelling was effected by borotritide reduction of red cells which had been treated with galactose oxidase.[712] This procedure led to extensive labelling of glycoproteins and glycolipids, whereas little labelling took place when the reactions were carried out on sealed, inside-out vesicles.

Cross-linking of two defined glycoproteins occurred when either cell ghosts or red cells reacted with dimethyl malonimidate (a cross-linking reagent).[713, 714]

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The electrochemical and biophysical properties of the red-blood cells of a patient with acquired polyagglutinability have been related to the reduced levels of sialic acid in the cell membranes, although other changes may also have occurred.[715]

No significant differences were apparent in the gel-electrophoretic patterns of proteins and glycoproteins from the membranes of red cells of patients with hereditary spherocytosis and those of controls.[716]

After modification by successive treatments with periodate ion and sodium borotritide, erythrocyte-membrane proteins exhibited less blood-group M activity, although the blood-group A and B activities and the binding sites for phytohaemagglutinin were not affected.[717]

A useful method for isolating human T-lymphocytes by means of red cells has been reported; the lymphocytes are obtained with a high degree of purity and with good recoveries.[718] Thus, pretreatment of sheep erythrocytes with neuraminidase facilitated the specific binding to, and the formation of rosettes with, human T-lymphocytes, which can be recovered following dissociation of the complex at 37 °C.

Thin-layer gel filtration has been used to examine antigens on red-blood cell membranes.[719] An extensive review of the properties of red-blood cells, including the nature and genetics of the blood-group substances, has appeared.[720]

An I-active substance has been isolated from the aqueous layer following extraction of human-erythrocyte membranes with butan-1-ol.[721] The substance also carried A, B, and H activities and contained 10% carbohydrate, none of which is sialic acid.

There has been further support for the association of HL-A antigens with red cells, which are reported (see p. 316) not to carry β_2-microglobulin.[722] Because of this, haemagglutinating antibodies cross-reacted with HL-A28 and HL-A2 antigens.

Sialic acid residues of erythrocyte membranes may be involved in controlling the lifetime of the cell. Enzymic removal of sialic acid residues from red cells *in vitro* furnished a material that was removed from the circulation with a half-life of about 20 hours.[723, 724] Glycopeptides prepared from young erythrocytes have been shown to contain a higher proportion of sialic acid and 2-amino-2-deoxy-D-galactose than do those from old erythrocytes.[725, 726] The distribution of sialic acid residues on red cells is influenced by the distribution of spectrin at the inner-membrane surface; this was clearly shown by the effects produced by purified antibodies against spectrin.[727]

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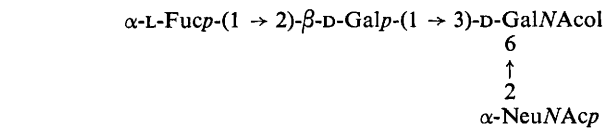
A glycoprotein, originally present in the membrane, has been isolated from sonicates of whole human platelets; it was shown to have a molecular weight of 1.48×10^5 and was converted into a second glycoprotein (mol. wt. 1.2×10^5) on treatment with trypsin.[728] Similar results were obtained with platelets from monkeys (*Erythrocebus patas*) and rabbits.[729] It is probable that sialic acid residues of glycoproteins are involved at the recognition site for, and in the absorption and active transport of, 5-hydroxytryptamine.[730] The nature of the glycoprotein binding site for thrombin on the surface of human platelets is not fully understood.[731]

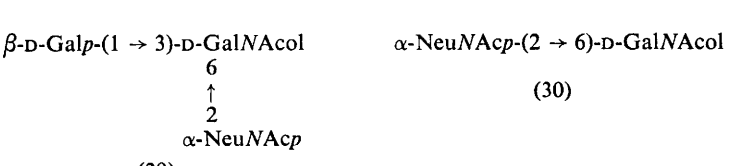
Human platelets catalysed the incorporation of D-mannose and 2-amino-2-deoxy-D-glucose into pre-existing protein *in vitro* by an energy-dependent process.[732] The glycoprotein was hydrolysed by thrombin.

Salivary, Mucous, and other Mammalian Body-fluid Glycoproteins

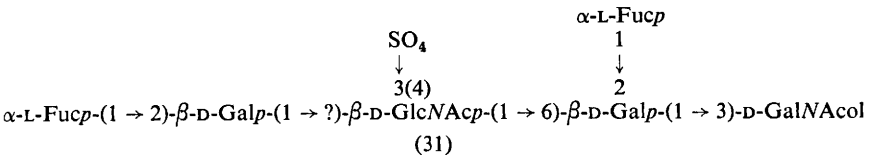
β-Elimination of oligosaccharides on treatment of proteins with alkali is a well-established technique for investigating the glycosidically linked carbohydrates of glycoproteins. With the glycoprotein from bovine submaxillary gland, the reaction occurred more effectively in a solvent composed of DMSO–water–ethanol (5 : 4 : 1) in the presence of 0.17M-KOH at 45 °C.[733] Alkaline borohydride reduction of canine submaxillary mucin gave a number of reduced oligosaccharides (28)—(34), none of which contains residues of both sialic acid and sulphated 2-acetamido-2-deoxy-D-glucose.[734] It appears that the requisite



(28)



(29) (30)



(31)

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$$\begin{array}{c} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad SO_4 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 3(4) \\ \alpha\text{-L-Fuc}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow ?)\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-D-Gal}N\text{Acol} \end{array}$$

(32)

$$\begin{array}{c} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad SO_4 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 3(4) \\ \beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-D-Gal}N\text{Acol} \end{array}$$

(33)

$$\begin{array}{c} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \alpha\text{-L-Fuc}p \\ SO_4 \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \downarrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ 3(4) \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2 \\ \beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-D-Gal}N\text{Acol} \end{array}$$

(34)

sialyl- and 2-acetamido-2-deoxy-D-glucosyl-transferases involved in the synthesis of the two types of chain have restricted specificities.

Human submaxillary mucin can be purified by precipitation from a salt solution with cetyltrimethylammonium bromide, followed by gel filtration; it has a molecular weight of $0.5—1.0 \times 10^6$ and is similar in amino-acid composition to mucins from other species.[735] The sugar composition is related to the ABO secretor status of an individual.

The glycoprotein inhibitor of β-glucuronidase present in porcine submaxillary gland has been found to differ from that present in submandibular gland inasmuch as it is much richer in 2-amino-2-deoxy-D-galactose but much poorer in 2-amino-2-deoxy-D-glucose.[736] The molecular weight (3.4×10^5) and amino-acid composition of each glycoprotein are similar, but not identical.[737] A similar inhibitor has been isolated from human saliva.[738]

The acylneuraminate citidyl- and sialyl-transferases from the submandibular glands of cows, pigs, and horses were able to utilize *N*-acetyl-, *N*-glycolyl-, *N*,7(8)-*O*-diacetyl-, *N*,4-*O*-diacetyl-, *N*-chloroacetyl-, and *N*-fluoroacetyl-neuraminic acids with equal facility.[739] The results confirmed the view that differences in the specificities of these enzymes cannot be used to explain why submandibular-gland glycoproteins from various animals differ in the type of sialic acid that they contain.

Water was taken up by sputum exposed to a water-saturated atmosphere, but is is not known whether or not this is due to the hydration of glycoproteins.[740] Mucous secretions of ephithelial goblet cells in explants of canine trachea contained glycoproteins that are relatively more sulphated than are those from submucosal glands.[741, 742]

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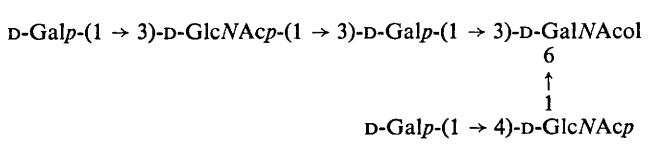
The levels of D-mannosyl-, D-galactosyl-, and L-fucosyl-transferases in lung microsomes were shown to be considerably higher in patients with cystic fibrosis than in those of controls.[743] It seems unlikely that this is the primary cause of cystic fibrosis, and they probably result indirectly from tissue atrophy. Cultured skin fibroblasts from patients with cystic fibrosis and from controls had closely similar levels of microsomal D-galactosyltransferase.[744]

Vitamin-A-deficient rats synthesized less of one or more L-fucose-containing glycoproteins present in the trachea, but the reason for this change is unknown.[745]

Ciliated nasal polyp epithelium taken from patients with cystic fibrosis incorporated relatively more sulphate than 2-amino-2-deoxy-D-glucose into glycoproteins *in vitro* by comparison with the amounts taken up by a control group.[746]

The glycoprotein from porcine gastric mucus can be freed from contaminating protein by equilibrium centrifugation in a gradient of caesium chloride,[747] which, even at low concentrations, causes irreversible changes in the conformation of the glycoprotein.[748] The structures of glycopeptides from porcine gastric mucosa have been investigated.[749]

Many preparations of porcine gastric glycoprotein have been found to contain a neuraminidase, probably derived from gut bacteria, which releases sialic acid from the glycoprotein under appropriate conditions of pH.[750] Human gastric, H-active mucin contains an oligosaccharide side-chain of structure (35).[751]



(35)

It is not known how 2-acetamido-2-deoxy-D-glucose kinase (mol. wt. 7.7×10^4), which has been isolated from human gastric mucosa, is involved in glycoprotein biosynthesis.[752] Extracts of human gastric mucosa solubilized with *N*-acetylcysteine and the mucin present therein yielded sulphated glycoproteins of closely similar (or identical) amino-acid and sugar compositions.[753]

The carbohydrate moieties of glycoproteins from sheep gastric mucosa have been shown to contain the terminal sequence L-Fucp-(1 → 2 or 6)-D-Galp-(1 → 6)-D-GlcNAcp or NeuNGlp-(2 → 6)-D-GalNAcp-(1 → 3)-(?)D-Galp.[754]

[743] P. Louisot and C. Levrat, *Clinica Chim. Acta*, 1973, **48**, 373.
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[745] F. Bonnani and L. De Luca, *Biochim. Biophys. Acta*, 1974, **343**, 632.
[746] T. F. Boat, J. I. Kleinerman, D. M. Carlson, W. H. Maloney, and L. W. Matthews, *Amer. Rev. Resp. Diseases*, 1974, **110**, 428.
[747] B. J. Starkey, D. Snary, and A. Allen, *Biochem. J.*, 1974, **141**, 633.
[748] D. Snary, A. Allen, and R. H. Pain, *Biochem. J.*, 1974, **141**, 641.
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[750] A. Allen and B. J. Starkey, *Biochim. Biophys. Acta*, 1974, **338**, 364.
[751] M. D. G. Oates, A. C. Rosbottom, and J. Schrager, *Carbohydrate Res.*, 1974, **34**, 115.
[752] A. Gindzieński, D. Głowacka, and K. Zwierz, *European J. Biochem.*, 1974, **43**, 155.
[753] J. Schrager and M. D. G. Oates, *Biochim. Biophys. Acta*, 1974, **372**, 183.
[754] R. Huguet, M. Solere, and N. Remy-Heintz, *Carbohydrate Res.*, 1972, **24**, 393.

Internal and brush-border membranes of cells of the small intestine of rats contain glycoproteins and glycolipids.[755] Biosynthesis of the glycoproteins occurs with a delay in the labelling of the rough membrane.[756]

Phenylbutazone (a potent anti-inflammatory drug) has been found to inhibit the incorporation of 2-amino-2-deoxy-D-glucose into glycoproteins synthesized by rat intestinal slices *in vitro*.[757] In this respect its action is similar to that of sodium salicylate. There appears to be no involvement of sulphated glycoproteins, which inhibit the proteolytic activity of pepsin, in the pathogenesis of duodenal ulcers.[758] The glycoproteins from bovine duodenal mucosa have been investigated.[759]

The molecular-weight distribution of porcine intestinal glycoproteins has been examined by ultracentrifugation under conditions of meniscus depletion,[760] and the general utility of this method was also demonstrated in investigations of a monodisperse glycoprotein present in the cyst fluid of the tapeworm *Cysticercus tenuicollis*.[761] Intestinal glycoproteins of germ-free rats have been shown to contain D-galactose, D-mannose, L-fucose, sialic acid, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose.[762]

Maltase and sucrase, which are located on the external surface of enterocyte membranes, have been identified as glycoproteins containing 45—50% and 21—30% of carbohydrate, respectively.[763] D-Xylose appears to be present in both enzymes, which exhibits ABO blood-group activity.

A vitamin-A-dependent, L-fucose-containing glycoprotein normally present in rat intestinal mucosa was also present in the media of cultures of the organ *in vitro*, and was probably derived from desquamated cells.[764]

Glycoproteins and glycosaminoglycans present in gall-bladder walls and in bile have been isolated, although examination of their structures and biosyntheses is needed.[765] Glycoproteins present in the bile of pigs, sheep, cattle, and humans have been shown to contain *NO*-diacetylneuraminic acids, and *O*-acetylated sialic acid residues are also present in the epithelial intestinal glycoproteins of rats and humans.[766] These sialic acids residues were hydrolysed relatively slowly, and they did not stain with the periodic acid–Schiff reagent unless the *O*-acetyl groups were removed.[767]

Mucosal biopsies of rabbit colon and human rectum maintained in culture have been shown to synthesize and to secrete glycoproteins.[768] By comparison

[755] Y. S. Kim and J. M. Perdomo, *Biochim. Biophys. Acta*, 1974, **342**, 111.
[756] Y. S. Kim and J. M. Perdomo, *F.E.B.S. Letters*, 1974, **44**, 309.
[757] B. E. Luke and G. G. Forstner, *Biochim. Biophys. Acta*, 1974, **338**, 345.
[758] F. André, D. Bouhours, C. André, F. Descos, and R. Lambert, *Clinica Chim. Acta*, 1974, **56**, 255.
[759] G. Cetta, G. Pallavicini, A. Calatroni, and A. A. Castellani, *Ital. J. Biochem.*, 1972, **21**, 275.
[760] R. A. Gibbons, S. N. Dixon, and D. H. Pocock, *Biochem. J.*, 1973, **135**, 649.
[761] S. N. Dixon, R. A. Gibbons, J. Parker, and R. Sellwood, *Internat. J. Parasitol.*, 1973, **3**, 419.
[762] J. K. Wold, T. Midtvedt, and R. Winsnes, *Acta Chem. Scand.*, 1973, **27**, 2997.
[763] J. J. Kelly and D. H. Alpers, *J. Biol. Chem.*, 1973, **248**, 8216.
[764] H. K. Kleinman and G. Wolf, *Biochim. Biophys. Acta*, 1974, **354**, 17.
[765] T. Terho and M. Laitio, *Biochim. Biophys. Acta*, 1974, **338**, 135.
[766] J. A. Cabezas and M. Ramos, *Carbohydrate Res.*, 1972, **24**, 486.
[767] C. F. A. Culling, P. E. Reid, M. G. Clay, and W. L. Dunn, *J. Histochem. Cytochem.*, 1974, **22**, 826.
[768] R. P. MacDermott, R. M. Donaldson, and J. S. Trier, *J. Clin. Invest.*, 1974, **54**, 545.

with controls, patients with active ulcerative colitis incorporated 2-amino-2-deoxy-D-glucose more extensively into glycoproteins, which were then rapidly secreted.

An acid-stable proteinase present in human cervical mucus is distinct from any of those present in serum.[769] The enzyme does not inhibit the acrosomal proteinase acrosin,[770] but it does inhibit proteinases present in large numbers in leucocytes after mating.[771] The mucus may function as a carrier of a protective enzyme.

Urinary Glycoproteins

The urinary excretion of antigens, many of which are glycoproteins but which are not derived from plasma glycoproteins, has been discussed in some detail.[772] The αM-foetoprotein (mol. wt. 5.7×10^5) was not detected in normal urine in pregnancy, but it was excreted by pregnant rats with an induced injury to the glomerular capillary wall.[773]

Tamm–Horsfall glycoprotein can be determined by radio-immunodiffusion methods.[774] C.d. measurements on the protein from humans and the asialo-derivative thereof showed that they possess little, if any, α-helicity, but a significant proportion of the β-structure is present. Gel-formation mediated by calcium ions was found to be inhibited in human Tamm–Horsfall asialoglycoprotein.[775] The protein excreted in hamster urine is devoid of sialic acid.[7]

Human hepatocyte membranes appear to contain a substance which cross-reacted immunologically with antisera raised in rabbits against human Tamm–Horsfall glycoprotein.[776]

Two closely related glycoproteins, neither of which was previously known, have been isolated from the urine of a patient with chronic myelocytic leukaemia.[777] One of the glycoproteins (mol. wt. 2.9×10^4) was shown to contain 61% of carbohydrate, whereas the other contains less carbohydrate but has an identical amino-acid composition.

An increased level of urinary excretion of proteoglycans has been noted and measured in a number of collagen diseases.[778]

The mean levels of excretion of follicle-stimulating hormone and luteinizing hormone by a group of males with a 47-XYY chromosomal pattern were found to be within the normal range.[779] The urinary levels of these hormones have been measured in normal and in subfertile or infertile males; the mean level of follicle-stimulating hormone excreted increased with the sperm concentration.[780]

[769] H. Fritz, B. Förg-Brey, E. Fink, M. Meier, H. Schiessler, and C. Schirren, *Z. physiol. Chem.*, 1972, **353**, 1943.
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[772] J. H. Boss, T. Dishon, A. Durst, and E. Rosenmann, *Israel J. Med. Sci.*, 1973, **9**, 490.
[773] E. Rosenmann, T. Dishon, E. Okon, J. H. Boss, and G. Ganem, *Experientia*, 1974, **30**, 551.
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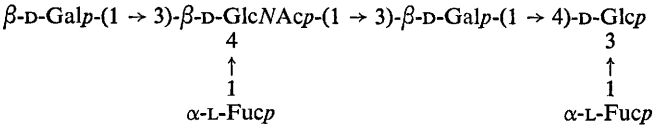
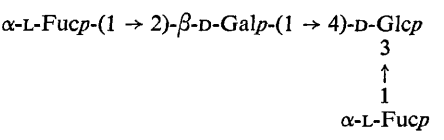
[776] D. C. Tsantoulas, I. G. McFarlane, B. Portman, A. L. W. F. Eddleston, and R. Williams, *Brit. Med. J.*, 1974, 491.
[777] D. Rudman, R. K. Chawla, A. E. Del Rio, and B. Hollins, *J. Clin. Invest.*, 1974, **53**, 868.
[778] H. W. Kreysel and A. Köhler, *Dermatologia*, 1974, **148**, 19.
[779] P. Christiansen and J. Nielsen, *Acta Endocrinol.*, 1973, **74**, 625.
[780] J. Mauss and G. Börsch, *Acta Endocrinol.*, 1973, **74**, 631.

The structures (36)—(43) of a number of oligosaccharides isolated from human urine have been elucidated.[781, 782] A sarcosine-containing glycopeptide, in which the carbohydrate chain is linked by way of D-xylose to a serine residue, has been isolated from the urine of patients with burns.[783] The tetrasaccharide (44) and the pentasaccharide (45) (60 mg of each in a litre of urine) were excreted by patients with mannosidosis.[784]



(36)



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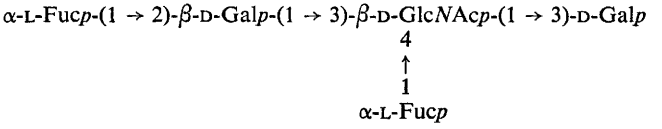

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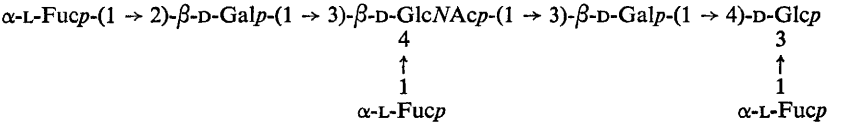

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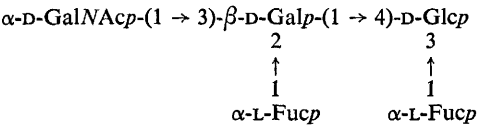

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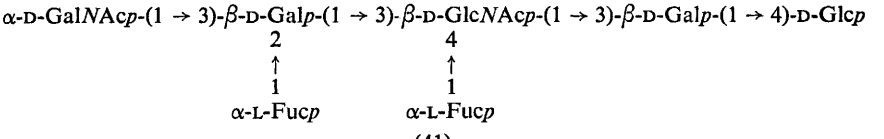

(41)

[781] G. Strecker and J. Montreuil, *Compt. rend.*, 1973, **277**, *D*, 1393.
[782] A. Lundblad and S. Svensson, *Carbohydrate Res.*, 1973, **30**, 187.
[783] D. K. Banerjee and D. Basu, *Indian J. Biochem. Biophys.*, 1974, **11**, 141.
[784] N. E. Nordén, A. Lundblad, S. Svensson, and S. Autio, *Biochemistry*, 1974, **13**, 871.

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

(42)

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

(43)

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-Glc*N*Ac*p*

(44)

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-Glc*N*Ac*p*

(45)

The estimation of D-xylose in blood and in urine, which forms part of a test for intestinal absorption, can be achieved by a modification of the Bial reaction; the method is applicable to 0.2—1.0 μg of D-xylose and requires only 20 μl of blood.[785] The levels of D-mannose, D-galactose, D-glucose, L-fucose, D-xylose, and D-ribose in urine can be assayed by gel chromatography, followed by g.l.c. of appropriately derivatized fractions.[786]

Avian-egg Glycoproteins

Ovalbumin mRNA comprises between 1670 and 2640 nucleotides, but only 1161 of these are needed for translation into ovalbumin.[787] There are about 3.6×10^4 molecules of ovalbumin mRNA present in each tubular gland cell of hens.[788] The half-life of this type of mRNA is about one day, but secondary stimulation of hens with oestrogen produced massive increases in the concentration of mRNA.[789]

Degradation of ovalbumin with L-ascorbic acid in the presence of copper(II) ions led to the appearance of a number of new, *N*-terminal amino-acids, the total amount being slightly in excess of 1 mole per mole of ovalbumin.[790]

The binding of ovalbumin to lysozyme was shown, by a fluorescence polarization method, to be predominantly electrostatic in nature, whereas hydrophobic forces dominate the binding of κ-casein to α_{s1}-casein.[791] The phosphate groups of ovalbumin were shown not to be involved in the binding of antibodies raised against the glycoprotein.[792]

[785] M. Rozental and L. Tomaszewski, *Clinica Chim. Acta*, 1974, **50**, 311.
[786] N. E. Norden, Ö. Eriksson, B. Hultberg, and P. A. Öckerman, *Clinica Chim. Acta*, 1973, **44**, 95.
[787] M. E. Haines, N. H. Carey, and R. D. Palmiter, *European J. Biochem.*, 1974, **43**, 549.
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[791] S. Nakai and C. M. Kason, *Biochim. Biophys. Acta*, 1974, **351**, 21.
[792] A. A. Ansari and A. Salahuddin, *Biochem. J.*, 1973, **135**, 705.

Thirteen oligosaccharides have been identified following acetolysis of avian ovomucoid glycopeptides.[793, 794]

A homogeneous glycoprotein closely similar to ovomucoid has been isolated from hen egg-white by a procedure that did not denature the material.[795] Its precise relation to ovomucoid is not clear.

The chiroptical properties of chicken ovomucoid and acetylated derivatives thereof have been examined at 297 and 77 K.[796]

A glycoprotein in the α_2-fraction of chicken sera cross-reacted immunologically with ovoinhibitor; these proteins are similar, but not identical, in a number of respects.[797] Three types of carbohydrate moiety occur in ovomucin; one contains equimolar proportions of D-galactose, 2-amino-2-deoxy-D-galactose, sialic acid, and sulphate, whereas the other is less complex.[798]

Duck ovotransferrin, which constitutes about 2% of the total solids of the egg-white, has been shown to be composed of a major and a minor component that can be separated by starch-gel electrophoresis.[799]

Phosvitin underwent conformational changes when it interacted with calcium or magnesium ions; at pH 6.5 and 25 °C, the glycoprotein can bind 127 calcium ions or 103 magnesium ions per mole, but these amounts were significantly reduced at pH 4.5.[800]

The half-life for the turnover of sulphated glycopeptides in egg-shell membrane and in magnum was found to be 1.2—1.5 days, whilst that of chondroitin sulphate in oviduct is 4 days.[801] The half-life (1.2 days) measured for the turnover of UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate confirmed that this glycosylated nucleotide does not function directly in the synthesis of sulphated glycoproteins.

Alkali-labile carbohydrate residues (mol. wts. 1100—2600) form part of a sulphated, glycoproteinaceous host factor (mol. wt. 2.6×10^4) isolated from chick allanotoic fluid; some of the sugar residues are esterified by fatty (C_{16}—C_{22}) acids.[802]

Miscellaneous Glycoproteins

A glycoprotein present in the yolks of grasshopper (*Locusta migratoria*) eggs constitutes 77% of the dry weight of the eggs; the carbohydrate moiety, probably linked to an asparagine residue, is composed of D-mannose (14) and 2-amino-2-deoxy-D-glucose (2 residues).[803]

The protein and carbohydrate moieties of the chorion of trout eggs have been examined by histochemical methods.[804]

[793] B. Bayard and J. Montreuil, *Carbohydrate Res.*, 1973, **24**, 427.
[794] B. Bayard, B. Fournet, S. Bouquelet, G. Strecker, G. Spik, and J. Montreuil, *Carbohydrate Res.*, 1973, **24**, 445.
[795] T. R. Oegema and G. W. Jourdian, *Arch. Biochem. Biophys.*, 1974, **160**, 26.
[796] E. Kay, E. H. Strickland, and C. Billups, *J. Biol. Chem.*, 1974, **249**, 793.
[797] A. J. Barrett, *Biochim. Biophys. Acta*, 1974, **371**, 52.
[798] A. Kato, K. Fujinaga, and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2479.
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[802] H. U. Choi and K. Meyer, *J. Biol. Chem.*, 1974, **249**, 932.
[803] K. Yamasaki, *Insect Biochem.*, 1974, **4**, 411.
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6
Enzymes

BY J. F. KENNEDY

Introduction

The purification of enzymes by affinity chromatography has now become an established technique that permits the isolation of enzymes by procedures much speedier and simpler than the classical methods of column chromatography. A general review of affinity chromatography discusses the principles involved, the choice of affinants, the characteristics of the adsorbents, and the conditions necessary for adsorption and for subsequent desorption of an enzyme.[1] Later sections of the review discuss the merits of affinity chromatography and the affinants commercially available. Another review specializes in the chemical aspects of affinity chromatography, discussion of which is given under the headings of 'designing the system', 'chemistry of the support matrix', and 'general ligands'.[2] A book on affinity chromatography, in addition to dealing with the chemistry and principles, describes group-specific adsorbents and their applications, and several special techniques.[3]

The mechanisms of the action of enzymes have been dealt with from the reaction viewpoint,[4] and a book entitled 'Enzyme Kinetics' provides an understanding of how a substrate 'saturates' an enzyme and how an analogue inhibits its action; discussions of allosteric phenomena and binding, and their implications for practical enzyme assays, are also included.[5]

The new volumes in the series 'Methods in Enzymology' deal with various aspects of the structures of enzymes in terms of molecular weight, conformations, transitions, interactions, and methods for the determination thereof.[6,7] The first volume in a new series on the electron microscopy of enzymes discusses the principles and methods of the technique.[8] A new volume in 'The Enzymes' series reviews the biosynthesis of enzymic proteins.[9]

A volume on enzymology in the practice of laboratory medicine contains a number of articles that fall within the general spheres of molecular biology,

[1] J. Turková, *J. Chromatog.*, 1974, **91**, 267.
[2] H. Guilford, *Chem. Soc. Rev.*, 1973, **2**, 249.
[3] C. R. Lowe and P. D. G. Dean, 'Affinity Chromatography', John Wiley, Chichester, 1974.
[4] M. Akhtar and D. C. Wilton, *Ann. Reports (B)*, 1972, **69**, 140.
[5] H. N. Christensen and G. A. Palmer, 'Enzyme Kinetics', 2nd edn., Saunders, Philadelphia, 1974.
[6] 'Methods in Enzymology', 'Enzyme Structure, Part C', ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1972, Vol. 26.
[7] 'Methods in Enzymology', 'Enzyme Structure, Part D', ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1973, Vol. 27.
[8] 'Electron Microscopy of Enzymes. Principles and Methods', ed. M. A. Hayat, Van Nostrand Reinhold, New York, 1973, Vol. 1.
[9] 'The Enzymes', ed. P. D. Boyer, Academic Press, New York, 1974, 3rd edn., Vol. 10.

analytical methodology, pathophysiology, and clinical interpretation of laboratory data.[10] An important article on the immunochemistry of enzymes covers the following aspects: the inhibition and enhancement of enzymic activity by specific antibodies, the antigenic determinants of enzymes, structure–function correlations pertaining to the catalytic and immunological activities, the immunological relationships between enzymes and their inactive precursors, apoenzymes, allosteric enzymes, multiple forms of enzymes, and the immunochemical characterization of enzymes.[11]

Newly reported automated assays for the determination of carbohydrase activity have been based on a modified ferricyanide reaction and the use of modular autoanalysers.[12] Fresh developments in the enzymic determination of D-glucose and its anomers have been reviewed.[13] An article on the inactivation of glycosidases describes the use of active-site-directed inhibitors to identify functional groups at the active site, thus permitting deductions on the mechanism of catalysis to be made.[14] Epoxide-type inhibitors appear to be especially suitable, since their reactivity is enhanced selectively by acid groups at the active site. In cases where a definite conclusion regarding the mechanism can be made, catalysis seems to occur *via* a carboxylate ion acting as a base and a carboxy-group acting as an acid. Hydrolysis probably proceeds by way of a substrate–enzyme intermediate, which may be a stabilized carbonium ion or a covalent intermediate.

The enzymic degradation of glycosphingolipids *in vivo* and *in vitro* has been reviewed; the various enzymes necessary to achieve complete breakdown were described.[15] Multiple forms of glycoside hydrolases have been reviewed, and their relation to known storage disorders involving the metabolism of glycolipids, glycoproteins, and glycosaminoglycans was discussed.[16] A subunit hypothesis was presented to explain the inter-relationships between the multiple forms and it may be of wide application to these enzymes. The abnormal enzymology of various lipidoses has been considered in the light of recent experiments with purified glycosidases, *etc.*, from human sources.[17] The structural, catalytic, and immunological properties of the enzymes involved were compared with those of their normal counterparts.

Immobilized forms of enzymes have continued to attract the attention of reviewers, and specialized aspects have been recounted.[18, 19] One review deals with such forms as a new type of active biological preparation,[20] whereas

[10] 'Enzymology in the Practice of Laboratory Medicine', ed. P. Blume and E. F. Freier, Academic Press, New York, 1974.
[11] R. Arnon, 'Immunochemistry of Enzymes', in 'The Antigens', ed. M. Sela, Academic Press, New York, 1973, Vol. 1, p. 85.
[12] K. Kainuma, K. Wako, A. Nogami, and S. Suzuki, *J. Jap. Soc. Starch Sci.*, 1973, **20**, 112.
[13] J. Okuda and I. Miwa, *Methods Biochem. Analysis*, 1973, **21**, 155.
[14] G. Legler, *Mol. Cell. Biochem.*, 1973, **2**, 31.
[15] R. W. Ledeen and R. K. Yu, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 105.
[16] D. Robinson, *Enzyme*, 1974, **18**, 114.
[17] J. F. Tallman, P. G. Pentchev, and R. O. Brady, *Enzyme*, 1974, **18**, 136.
[18] J. F. Kennedy, in 'Connective Tissues, Biochemistry, and Pathophysiology', ed. R. Fricke and F. Hartmann, Springer-Verlag, Berlin, 1974, p. 146.
[19] J. F. Kennedy, *Chem. Soc. Rev.*, 1973, **2**, 355.
[20] A. Krakowiak, K. Stachowicz, and J. Malanowska, *Przem. Ferment. Rolny*, 1973, **17**, 3.

another brief review describes the preparation, activity, kinetics, resistance to denaturation, and the utilization of immobilized enzymes in synthesis, analysis, biochemistry, medicine, and alimentation.[21] Enzymes immobilized on carbohydrates have been reviewed as chemically reactive derivatives of polysaccharides.[22] Analytical uses of immobilized enzymes have been summarized.[23]

The properties, kinetics, and applications of immobilized enzymes have been considered from the viewpoint of biochemical engineering, immobilized enzymes being regarded as an alternative to either whole cells or free enzymes.[24] The article also discusses environmental effects on the kinetic behaviour of immobilized enzyme systems, the performance of columns of immobilized enzymes, and the stability of these enzyme derivatives. The feasibility of gel-entrapment of enzymes as a possible means for the correction of inborn errors of metabolism has been tested, but the studies have raised serious questions as to the potential usefulness of this approach.[25]

Acetamidodeoxygalactosidases, Acetamidodeoxyglucosidases, and Acetamidodeoxyhexosidases

The purification of β-D-acetamidodeoxyglucosidases by affinity chromatography has been described.[26] Polysaccharide-support matrices were employed, and, in one approach, such inhibitors as 4-aminobenzyl and 4-aminophenyl 1-thioglycosides were used; in another approach, glycoproteins and glycopeptides were employed as ligands, the protein moieties thereof serving as spacer arms.

In an enzymological approach to the lipidoses, the involvement of β-D-acetamidodeoxyhexosidases A and B in the chemical defect underlying Tay–Sachs disease has been reviewed.[17] Other reviews of the deficiency of β-D-acetamidodeoxyhexosidase in Tay–Sachs disease have dealt with various aspects of the storage substance and the levels of lysosomal enzymes.[27, 28]

Cellulose acetate electrophoresis of extracts of various human tissues consistently showed, in addition to β-D-acetamidodeoxyhexosidases A and B, a faster-moving band with the same activity (designated β-D-acetamidodeoxyhexosidase C).[29] The relative intensity of the 'C' band was greater in extracts from embryonic tissues than from adult tissues. The characterization of β-D-acetamidodeoxyhexosidase C from human sources, including tissues from patients with Tay–Sachs or Sandhoff's disease, showed that the enzyme possesses a pH optimum of 7.8, compared with that of 5.3 for the B enzyme; the inter-relationships of the A, B, and C enzymes were discussed.[30] A further article on β-acetamidodeoxyhexo-

[21] E. Brown and A. Racois, *Bull. Soc. chim. France*, 1974, 743.
[22] J. F. Kennedy, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 305.
[23] G. G. Guilbault, ref. 10, p. 203.
[24] S. Aiba, A. E. Humphrey, and N. F. Millis, 'Biochemical Engineering', 2nd edn., Academic Press, New York, 1973.
[25] H. L. Nadler and S. J. Updike, *Enzyme*, 1974, **18**, 150.
[26] R. J. Sturgeon, *L'Actualité chimique*, No. 7, 1974, 38.
[27] K. Sandhoff and K. Harzer, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 346.
[28] J. S. O'Brien, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 323.
[29] L. Poenaru and J. C. Dreyfus, *Clinica Chim. Acta*, 1973, **43**, 439.
[30] I. Braidman, M. Carroll, N. Dance, D. Robinson, L. Poenaru, A. Weber, J. C. Dreyfus, B. Overdijk, and G. J. M. Hooghwinkel, *F.E.B.S. Letters*, 1974, **41**, 181.

sidases and Tay–Sachs disease has developed an earlier suggestion that the A and B enzymes possess subunits (β) in common, and that the A form, at least, must have specific subunits (α) determined by a separate locus such that A is $(\alpha\beta)_n$ and B is either $(\beta_2)_n$ or $(\beta\gamma)_n$ (γ representing a polypeptide subunit determined by a third locus).[31] The defect in Tay–Sachs disease would represent a mutation causing a diminished production of α-chains, whereas that in Sandhoff's disease would represent a mutation of the β-chain. Comments were made on immunological and amino-acid analyses supporting these suggestions. Studies of hybrid-cell clones played a useful part in assessing the enzyme defects, and the role of the stimulatory factor for β-D-acetamidodeoxyhexosidase B was discussed.

Extracts of cultured fibroblasts of normal human skin released the label from a heptasaccharide prepared by enzymic addition of ^{14}C-labelled 2-acetamido-2-deoxy-D-galactose to a hexasaccharide derived from chondroitin 4-sulphate, *viz.* *O*-β-D-glucopyranuronosyl-{(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl 4-sulphate)-(1 → 4)-*O*-β-D-glucopyranuronosyl)}$_2$-2-acetamido-2-deoxy-D-galactose 4-sulphate.[32] However, the fibroblasts from patients with either Tay–Sachs or Sandhoff–Jatzkewitz disease did not release labelled material from the substrate, suggesting that β-D-acetamidodeoxyhexosidase A is responsible for the activity against the heptasaccharide. Various other diseases were investigated for β-D-acetamidodeoxyhexosidase activity.

In a study of the activities of glycosaminoglycan hydrolases of normal and atherosclerotic human aorta, it was found that the β-D-acetamidodeoxyhexosidase activity could be correlated with the various stages of atherosclerosis.[33] Human aortas possess multiple forms of β-D-acetamidodeoxyhexosidase, which were separated by gel electrophoresis into the A and B forms.[31] The A and B forms were subdivided into five and eight bands, respectively, by isoelectric focusing, but only the B form was thermally stable.[34] All forms of the enzyme were active against 7-bromo-3-hydroxy-2-naphth-*o*-anisidinyl 2-acetamido-2-deoxy-β-D-glucopyranoside and the corresponding D-galactoside (activity ratio 1 : 8), suggesting that each form of the enzyme possesses two activities. It was found by heat inactivation that the aortic enzyme comprises 51% of the A form and 49% of the B form; neither form was affected by the action of neuraminidase. Whereas the B form was stable in purified form, the purified A form was gradually transformed into the B and other forms during storage at −20 °C. The β-D-acetamidodeoxyhexosidases of bovine epididymis were also active against the two glycosides, and were separated (isoelectric focusing) into thirteen bands having pI values more alkaline than those of the B forms from human sources.

β-D-Acetamidodeoxyglucosidase activities have been detected in human liver, placenta, urine, and blood platelets.[35]

A crude β-D-acetamidodeoxyhexosidase fraction from human liver and urine was able to convert Tay–Sachs ganglioside G_{M_2} into ganglioside G_{M_3} (see Vol. 7,

[31] A correspondent, *Nature*, 1974, **244**, 612.
[32] J. N. Thompson, A. C. Stoolmiller, R. Matalon, and A. Dorfman, *Science*, 1973, **181**, 866.
[33] D. Platt, in 'Connective Tissues and Ageing', ed. H. G. Vogel, Excerpta Medica, Amsterdam, 1973, p. 79.
[34] K. Hayase, S. R. Reisher, and B. F. Miller, *Prep. Biochem.*, 1973, **3**, 221.
[35] F. E. Brot, J. H. Glaser, K. J. Roozen, and W. S. Sly, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1.

p. 360).[36] This ability was shown to be due to the A enzyme and not to the B enzyme, and a heat-stable, non-dialysable preparation obtained from the crude fraction stimulated the conversion. However, extensive purification and ageing reduced the capacity of the enzyme to hydrolyse G_{M_2}, even in the presence of the heat-stable preparation, thus explaining why the A form has been reported by other investigators to hydrolyse G_{M_2} only with great difficulty. The increased storage of G_{M_2} has been related to the absence of the A enzyme in the classical form of Tay–Sachs disease. Ion-exchange chromatography of human-liver β-D-acetamidodeoxyhexosidase gave three peaks, designated A_1, A_2, and B, exhibiting activity.[37] An activator was required for the hydrolysis of glycosphingolipids by the enzyme. The activator was purified from liver, and stimulated the hydrolysis of the gangliosides G_{M_1} and G_{M_2} and ceramide trihexoside by the β-D-galactosidase, β-D-acetamidodeoxyhexosidase, and α-D-galactosidase of human liver, respectively. The requirement of a heat-stable activator for the glycosidases to hydrolyse the carbohydrate chains of glycosphingolipids suggests that the catabolism of these substrates is complex. The β-D-acetamidodeoxyhexosidase of human liver has been separated from a 'chitobiase' by ion-exchange chromatography and gel filtration using di-*N*-acetylchitobiose as substrate; the enzyme hydrolysing this substrate possesses a low molecular weight and was active towards 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[38]

The activity of β-D-acetamidodeoxyglucosidase in biopsy specimens of rectal mucosae from normal subjects and cystinotic children and their parents (pH optimum 4.5, K_m for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 0.67 mmol l^{-1}) has been determined.[39] A close correlation between the β-D-acetamidodeoxyglucosidase, β-D-galactosidase, and β-D-glucuronidase activities was found only in control specimens.

Following separation by ion-exchange chromatography, β-D-acetamidodeoxyhexosidases A and B from human placenta have been further purified by affinity chromatography on 2-acetamido-*N*-(6-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine coupled to a cyclic imidocarbonate derivative of macroporous agarose.[40] The enzyme forms were studied kinetically; both forms were inhibited competitively by the ligand and had identical K_i values. β-D-Acetamidodeoxyhexosidase A has been purified from human placental lysosomes by treatment of an extract with sodium deoxycholate, followed by ion-exchange chromatography and precipitation.[41] Rabbit antibodies raised to the A enzyme totally inactivated both the A and B enzymes, but the tissue extracts were only partly inactivated. Cellulose acetate electrophoresis showed that the residual activity corresponded to the C enzyme (see p. 330), indicating immunological differences between the A and C enzymes. Other immunological experiments showed that anti-β-D-acetamidodeoxyhexosidase A cross-reacted with the B enzyme, and *vice versa*.[42] Although a specific anti-A serum was obtained, the anti-B component could not

[36] Y.-T. Li, M. Y. Mazzotta, C.-C. Wan, R. Orth, and S.-C. Li, *J. Biol. Chem.*, 1973, **248**, 7512.
[37] S.-C. Li, C.-C. Wan, M. Y. Mazzotta, and Y.-T. Li, *Carbohydrate Res.*, 1974, **34**, 189.
[38] J. L. Stirling, *F.E.B.S. Letters*, 1974, **39**, 171.
[39] M. Genel, P. Holtzapple, O. Koldovsky, and S. Segal, *Clinica Chim. Acta*, 1973, **46**, 247.
[40] B. Geiger, Y. Ben-Yoseph, and R. Arnon, *F.E.B.S. Letters*, 1974, **45**, 276.
[41] L. Poenaru and A. Weber, *Biomedicine*, 1973, **19**, 538.
[42] E. Beutler and S. K. Srivastava, *Israel J. Med. Sci.*, 1973, **9**, 1335.

be purified. Related studies were also carried out on enzymes obtained from patients with Tay–Sachs or Sandhoff's disease. It was concluded that each enzyme represents an octamer comprising two types of subunit. Thus, the A enzyme was designated as $(\alpha\beta)_4$ and the B enzyme as $(\beta\gamma)_4$. The presence of a common subunit provides an explanation for the disappearance of both enzyme activities in Sandhoff's disease. A model based on three genetic loci fits the data and is consistent with studies of cell hybridization. Amino-acid analyses of the A and B enzymes have shown that the *N*-terminus of each is blocked. Further studies on human placental β-D-acetamidodeoxyhexosidases involving reactions with antibodies, treatment with neuraminidase, and immunoelectrophoresis are compatible with a number of models for the enzyme, but it seems unlikely that the B form is a 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic-acid-free derivative of the A form.[43] It is more likely that both forms share a common unit. Sandhoff's disease can then be ascribed to a mutation affecting a subunit present only in β-D-acetamidodeoxyhexosidase A, which might contain two different subunits $(\alpha\beta)_n$, whereas the B enzyme contains only one of these types of subunit $(\beta\beta)_n$. This would explain the cross-reactivity of antisera produced against both forms of the enzyme, and the fact that specific anti-A sera, but not specific anti-B sera, can be produced. It also explains the increased amount of the B enzyme found in Tay–Sachs disease, since uncombined β-subunits would be available.

The major β-D-acetamidodeoxyglucosidase (pI 4.73, molecular weight 1.05×10^5) in human blood plasma has been isolated and its purity was established by gel filtration and isoelectric focusing.[44] The physicochemical characteristics remained unchanged after reduction and carboxymethylation of the cysteinyl residues, even in concentrated solutions of guanidinium hydrochloride. The enzyme contains no free thiol groups, but comprises sialic acid (1.4), D-glucose and D-galactose (together 30.0), and 2-amino-2-deoxy-D-glucose (3.5 residues per molecular weight of 10^5); L-fucose was not detected. Kinetic studies indicated that the enzyme also possesses β-D-acetamidodeoxygalactosidase activity (pH optimum 4.5—5.0). The enzyme was inhibited by Cu^{2+}, Hg^{2+}, and Ag^+ ions and ascorbate; the strong inhibition by Hg^{2+} and Ag^+ ions was partly reversible. The β-D-acetamidodeoxyhexosidase of human serum has been separated into two forms by ion-exchange chromatography.[45] The minor form possessed properties identical with those of the β-D-acetamidodeoxyhexosidase of human liver, whereas the properties of the other form were different. Neuraminidase hydrolysed the major component, but neither the minor component nor the liver enzyme was affected. The β-D-acetamidodeoxyhexosidase A from tears is similar to the serum A enzyme, whereas the A enzyme from urine and lymph is similar to the liver enzyme. It was suggested that the A enzyme of serum might be derived by glycosylation of the liver enzyme before secretion.

Polyacrylamide gel electrofocusing of sera from normal and pregnant subjects and from cases of Tay–Sachs disease, cancer, and hyperlipidemia separated the β-D-acetamidodeoxyhexosidase activity into a number of isoenzymes.[46] The

[43] S. K. Srivastava and E. Beutler, *Nature*, 1973, **241**, 463.
[44] J. A. Verpoorte, *Biochemistry*, 1974, **13**, 793.
[45] J. U. Ikonne and R. B. Ellis, *Biochem. J.*, 1973, **135**, 457.
[46] K. Hayase and D. Kritchevsky, *Clinica Chim. Acta*, 1973, **46**, 455.

A, B, and P forms of the enzyme, and their thermal stabilities and band-transformation phenomena, were investigated. Elevation of the level of β-D-acetamidodeoxyhexosidase activity during pregnancy was attributed to a progressive increase in the levels of a number of isoenzymes, and it was suggested that the P form may occur specifically during pregnancy.

Elevated levels of serum β-D-acetamidodeoxyglucosidase have been found in some cases of rheumatoid arthritis.[47] A correlation was indicated between the β-D-acetamidodeoxyglucosidase activity of sera and the lysosomal enzymes in the inflamed tissues. Since serum β-D-acetamidodeoxyhexosidase can be inactivated by brief treatment at pH 2.8 and 37 °C, an assay has been devised for carrier-detection tests for Tay–Sachs disease.[48] Activity levels of β-D-acetamidodeoxyglucosidase for sera from normal, carrier, pregnant, and Tay–Sachs subjects were reported, and the results of inactivation by acid and heat correlated well. A 15-fold increase in β-D-acetamidodeoxyglucosidase activity in a patient with I-cell disease (mucolipidosis II) was shown to reside in two enzyme forms exhibiting electrophoretic behaviour similar to that normally seen in control sera.[49] The greater increase (28-fold) was in the heat-stable form of the enzyme. By contrast, the decreased activity in fibroblast cells from the same patient was associated with multiple forms of β-D-acetamidodeoxyglucosidase, clearly distinguishable from those seen in sera or fibroblast cells of the controls. Using the levels of β-D-acetamidodeoxyglucosidase activity in sera and leucocytes as a basis for expression of the α-D-mannosidase activity, it has been possible to distinguish cases of mannosidosis and related heterozygotes.[50]

A correlative study of the β-D-acetamidodeoxyglucosidase and α-D-mannosidase activities of human cerebrospinal fluid has been performed using a spectrofluorometric assay.[51]

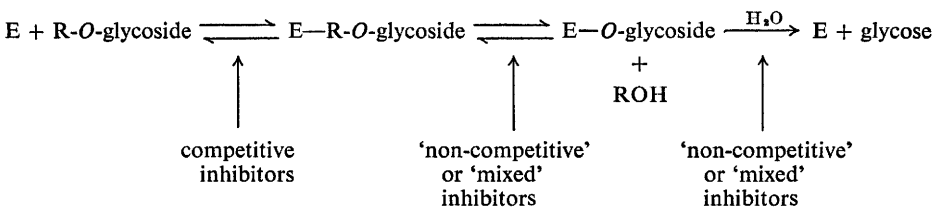
Human urinary β-D-acetamidodeoxyglucosidase has been purified by affinity chromatography on an agarose matrix to which 4-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside had been coupled.[52] Although the enzyme was still impure, it consisted of the A form, since chromatography removed the B form.

The β-D-acetamidodeoxyhexosidase of equine kidney has been separated into three forms (A, A′, and B) by ion-exchange chromatography; the three forms exhibited the following properties: isoelectric points 6.07, 6.25, and 8.20; molecular weights 1.25×10^5, 2.5×10^5, and 1.25×10^5; and K_m values of 0.147, 0.102, and 0.043 mmol l^{-1} with phenyl 2-acetamido-2-deoxy-β-D-galactopyranoside, and 0.571, 0.246, and 0.086 mmol l^{-1} with 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside, respectively.[53] The A form was labile to heat, whereas the B form was not. Oligosaccharides from ganglioside G_{M_1} were hydrolysed by the A and A′ forms, whereas those from asialo-G_{M_1} and globoside I were hydrolysed

[47] N. C. Kar and C. M. Pearson, *Proc. Soc. Exp. Biol. Med.*, 1973, **142**, 398.
[48] A. Saifer and A. L. Rosenthal, *Clinica Chim. Acta*, 1973, **43**, 417.
[49] K. K. Lie, G. H. Thomas, H. A. Taylor, and J. A. Sensenbrenner, *Clinica Chim. Acta*, 1973, **45**, 243.
[50] P. K. Masson, A. Lundblad, and S. Autio, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 296.
[51] J. L. Viallard, C. Motta, and G. Dastugue, *Pathol. Biol.*, 1973, **21**, 973.
[52] E. E. Grebner and I. Parikh, *Biochim. Biophys. Acta*, 1974, **350**, 437.
[53] Y. Seyama and T. Yamakawa, *J. Biochem. (Japan)*, 1974, **75**, 495.

by all forms. Treatment of the A form with neuraminidase did not yield the B form. Relations among the multiple forms of the β-D-acetamidodeoxyhexosidase of equine kidney were investigated by electrophoresis on Cellogel; the A and B forms remained unaltered, but the A′ form yielded a mixture of the other forms. It was concluded that the A′ form is a complex of the A and B forms.[54]

Most of the β-D-acetamidodeoxyhexosidase activity of a homogenate of bovine epididymis was found to occur in the soluble fraction, polyacrylamide gel electrophoresis of which gave 12 active bands.[55] Both soluble and cellular enzyme activities against 7-bromo-3-hydroxy-2-naphth-*o*-anisidinyl 2-acetamido-2-deoxy-β-D-glucopyranoside were inhibited by 2-acetamido-2-deoxy-D-glucono-1,4(5)-lactone. It was shown that treatment of the cellular enzyme with neuraminidase caused several of the fast-moving electrophoretic bands to disappear. β-D-Acetamidodeoxyhexosidase from commercial bovine serum albumin was inhibited by D-*glycero*-D-*gulo*-heptono-1,4-lactone, D- and L-glucono-1,5-lactones, D-galactono-1,5-lactone, D-fucono-1,5-lactone, L-arabinono-1,4-lactone, D-ribono-1,4-lactone, L-mannono-1,4-lactone, and L-ascorbic acid.[56] The kinetics of inhibition of the hydrolysis of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galacto- and -gluco-pyranosides by the enzyme were examined in detail. Both D-glucono-1,5-lactone and L-ascorbic acid gave mixed-type inhibitions with the latter substrate, whereas they were non-competitive inhibitors of the D-galactoside; lactones derived from 2-acetamido-2-deoxy-D-galactose and -glucose were competitive inhibitors for both glycosides. The lactones are considered to act at different stages in the sequence shown in Scheme 1.



Scheme 1

Ovine-brain β-D-acetamidodeoxyhexosidase has been found to bind with concanavalin A to form an enzymically active precipitate.[57] The activity of the complex was inhibited by methyl α-D-glucopyranoside, which, depending on the pH of the medium, also dissociated the complex; maximum dissociation occurred at pH 4.0. Since the glycoside differentially dissociated the complexes formed from other enzymes and the phytohaemagglutinin, complex formation could be used to purify the β-D-acetamidodeoxyhexosidase from a mixture of enzymes. The enzyme is assumed to be a glycoprotein.

[54] Y. Seyama and T. Yamakawa, *J. Biochem.* (*Japan*), 1974, **75**, 947.
[55] K. Hayase, S. R. Reisher, and D. Kritchevsky, *Proc. Soc. Exp. Biol. Med.*, 1973, **142**, 466.
[56] J. N. Kanfer and C. H. Spielvogel, *Biochim. Biophys. Acta*, 1973, **327**, 405.
[57] S. Bishayee and B. K. Bachhawat, *Biochim. Biophys. Acta*, 1974, **334**, 378.

The β-D-acetamidodeoxyhexosidase components of ram testis and epididymis have been separated by ion-exchange chromatography.[58] Although all components possess the same molecular weight (1.4×10^5) and similar catalytic activity towards 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides, isoelectric focusing of the components showed that each possesses a distinct pI value. Isoelectric focusing has also been used to demonstrate the presence of multiple forms of the enzyme in ram semen.

Isoelectric focusing has been used to separate other enzymes from a β-D-acetamidodeoxyhexosidase present in porcine kidney.[59]

α-D-Acetamidodeoxyglucosidase and β-D-acetamidodeoxy-galactosidase and -glucosidase activities have been found in a variety of invertebrates from the Sea of Japan.[60] The distribution of these glycosidases was reported for selected species of Coelenterata, Annelida, Arthropoda, Mollusca, Echinoderma, and Chordata.

The β-D-acetamidodeoxyglucosidase of germinating seeds of *Canavalia ensiformis* has been purified by gel filtration and ion-exchange chromatography,[61] and those of Jack-bean meal and wheat-germ agglutinin have been separated from other glycoside hydrolases by affinity chromatography on an agarose derivative with a spacer arm to which 4-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside had been coupled.[62]

In a study of the *N*-acyl specificity of the β-D-acetamidodeoxyglucosidase present in 'Taka amylase' (a crude α-amylase preparation from *Aspergillus oryzae*), it was found that 4-nitrophenyl 2-deoxy-2-methylsulphonamido-β-D-glucopyranoside was not hydrolysed, even at high concentrations of the enzyme.[63] This glycoside inhibited the hydrolysis of the corresponding *N*-acetyl derivative, indicating that the two glycosides bind to the same site of the enzyme. It was concluded that the carbonyl group of the *N*-acetyl derivative is essential for the manifestation of enzymic activity, and that it cannot be replaced with other functional groups. The carbonyl group is thought to be involved in neighbouring-group participation in the enzymic reaction.

In a study of the control of the production of β-D-acetamidodeoxyglucosidase by *Bacillus subtilis* grown on a chemically defined medium, derepression was observed during glucogenesis and during the initial stages of sporulation.[64] β-D-Acetamidodeoxyhexosidase was shown to be one of the enzymes involved in the sequential actions of cell-wall hydrolases in the germination and outgrowth of *Microsporum gypseum* macroconidia.[65] Together with other hydrolases, the enzyme is localized in lysosomal vesicles, which appeared to be delivered to the germinating spores in a co-ordinated manner.

[58] S. Bullock and B. Winchester, *Biochem. J.*, 1973, **133**, 593.
[59] G. Y. Wiederschain, *Doklady Akad. Nauk S.S.S.R.*, 1974, **214**, 462.
[60] N. V. Molodtsov, M. G. Vafina, A. Kim, E. V. Sundukova, A. A. Artyukov, and Y. G. Blinov, *Comp. Biochem. Physiol.*, 1974, **48B**, 463.
[61] A. Boersma and P. Degand, *Compt. rend.*, 1974, **278**, *D*, 1903.
[62] M. E. Rafestin, A. Obrenovitch, A. Oblin, and M. Monsigny, *F.E.B.S. Letters*, 1974, **40**, 62.
[63] K. Yamamoto, *J. Biochem.* (*Japan*), 1974, **75**, 931.
[64] S. J. Brewer and R. C. W. Berkeley, *Biochem. J.*, 1973, **134**, 271.
[65] W. J. Page and J. J. Stock, *Canad. J. Microbiol.*, 1974, **20**, 483.

During autolysis of *Sclerotinia fructigena*, the culture filtrates contained increased quantities of a β-D-acetamidodeoxyglucosidase.[66] After purification by gel filtration and isoelectric focusing, the enzyme gave a single band (pI 3.76) on cellulose acetate electrophoresis. Although the enzyme did not readily hydrolyse either chitin or a glycopeptide containing terminal 2-acetamido-2-deoxy-D-glucosyl residues, it rapidly degraded di-*N*-acetylchitobiose. The enzyme (K_m 2.0 mmol l^{-1} using 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside) was less susceptible to inhibition by lactones than the corresponding enzyme from other sources; inhibition by an excess of the substrate was observed. The culture filtrate also contained β-D-acetamidodeoxygalactosidase activity, and 2-acetamido-2-deoxy-D-glucose acted as a source of nitrogen for the organism during the production of the enzyme.

Arabinofuranosidases

α-L-Arabinofuranosidase was secreted by *Sclerotinia fructigena* grown on a medium containing pectin; the enzyme was easily liberated from the mycelia, although it was labile at low pH.[67] However, a particulate form of the enzyme was recovered from a density gradient of sucrose in a fraction that also contained mitochondria. Evidence was obtained for a structure-linked latency of the enzyme and an acid phosphatase also present, and for the secretion of the enzyme by a process of reverse pinocytosis. The production of both intra- and extracellular α-L-arabinofuranosidases by *S. fructigena* has been confirmed; the two enzymes differ in their kinetic parameters, pH stabilities, and pH optima (4.5 and 5.5—6.0, respectively).[68] Both enzymes gave two peaks on gel filtration; maximum activity of the extracellular enzyme was associated with the peak of lower molecular weight, whereas the peak of higher molecular weight was more active in the mycelial homogenate. Isoelectric focusing of the filtrate gave two peaks of activity (pI 3.0 and 6.5), maximum activity being associated with the latter, but most of the activity in the mycelial homogenate was associated with a third peak (pI 4.5). Physical and kinetic data indicated that forms with the same pI value from the two sources are identical.

The α-L-arabinofuranosidase detected in apple fruit infected with *Sclerotinia fructigena* yielded three isoenzymes on preparative isoelectric focusing, and the isoenzyme pattern was similar to that obtained from cultures of the organism grown on various carbon sources, including sodium polypectate.[69] Although each isoenzyme showed specificity for α-L-arabinofuranosides, they differed in substrate specificities and towards inhibitors. Both extracellular enzymes released L-arabinose from arabinan and the cell walls of apples. Antiserum to the purified α-L-arabinofuranosidase was shown to be specific by immunodiffusion, by immunoadsorption on an anti-enzyme column, and by the binding of fluorescein-conjugated serum to the antigen.[70]

[66] F. Reyes and R. J. W. Byrde, *Biochem. J.*, 1973, **131**, 381.
[67] E. C. Hislop, V. M. Barnaby, C. Shellis, and F. Laborda, *J. Gen. Microbiol.*, 1974, **81**, 79.
[68] F. Laborda, A. H. Fielding, and R. J. W. Byrde, *J. Gen. Microbiol.*, 1973, **79**, 321.
[69] F. Laborda, S. A. Archer, A. H. Fielding, and R. J. W. Byrde, *J. Gen. Microbiol.*, 1974, **81**, 151.
[70] E. C. Hislop, C. Shellis, A. H. Fielding, F. J. Bourne, and J. W. Chidlow, *J. Gen. Microbiol.*, 1974, **83**, 135.

β-D-Fructofuranosidases

It appears that β-D-fructofuranosidase activity decreases with continuous and repeated use of the enzyme, partly because of alterations in the tertiary structure of the enzyme, but not necessarily because of the effects of shear, adsorption, and polarization.[71] The studies suggested that the use of a very stable environment (including temperature, pH, and water, ion, salt, and enzyme concentrations, the hydration number of the enzyme and the substrate, buffer, stabilizing agents, and surface-active agents, *etc.*) for a given enzyme reaction is of the utmost importance for the continuous operation of the enzyme system under optimal conditions.

The localization of β-D-fructofuranosidase in canine kidney has been studied by a multiple-indicator dilution technique with [^{14}C]sucrose as the substrate.[72]

The molecular weight (2.2×10^5) and number of subunits of the β-D-fructofuranosidase–oligo-1,6-D-glucosidase complex from rabbit small intestine have been determined by gel filtration, polyacrylamide gel electrophoresis in sodium dodecyl sulphate, and fingerprint analysis of tryptic hydrolysates.[73] The complex comprises two subunits of similar size (molecular weights $1.1—1.2 \times 10^5$). The complex and 'inactive β-D-fructofuranosidase' contain D-galactose (11, 8), D-mannose (12, 14), D-glucose (5, 7), L-fucose (8, 9), 2-amino-2-deoxy-D-galactose (5, 6), and 2-amino-2-deoxy-D-glucose (31, 34 units, respectively, for a molecular weight of 10^5).

A study of β-D-fructofuranosidase and αα-trehalase activities of hamster small intestine has confirmed the presence of a disaccharidase-associated transport system different from the monosaccharide-transport system dependent on sodium ions.[74] Both D-glucose and D-fructose released from sucrose were transferred directly, although the extent of uptake is unrelated to the total β-D-fructofuranosidase activity. It was suggested that the brush-border disaccharidases may subserve a translocating 'carrier' function for part of the products of the enzyme's action.

The development of β-D-fructofuranosidase in the liver and intestine of chick embryos and chicks has been studied, and the effect of administration of hydrocortisone upon the levels in the embryo was examined.[75]

Isolated cell walls of *Convolvulus arvensis* callus have been shown to contain an 'acid' β-D-fructofuranosidase (pH optimum 4.5—4.8) activity, but none of the corresponding 'neutral enzyme'.[76] The β-D-fructofuranosidase activity increased significantly on incubation of callus fragments in nutrient solution.

Two inducible enzymes, a β-D-fructofuranosidase and a levansucrase, have been shown to be responsible for the saccharolytic activity of a strain of *Bacillus subtilis*.[77]

[71] L. Bowski and D. Y. Ryu, *Biotechnol. Bioeng.*, 1974, **16**, 697.
[72] M. Silverman, *J. Clin. Invest.*, 1973, **52**, 2486.
[73] A. Cogoli, A. Eberle, H. Sigrist, C. Joss, E. Robinson, H. Mosimann, and G. Semenza, *European J. Biochem.*, 1973, **33**, 40.
[74] K. Ramaswamy, P. Malathi, W. F. Caspary, and R. K. Crane, *Biochim. Biophys. Acta*, 1974, **345**, 39.
[75] C. F. Strittmatter, *Enzyme*, 1973, **15**, 24.
[76] F. M. Klis, R. Dalhuizen, and K. Sol, *Phytochemistry*, 1974, **13**, 55.
[77] M. Pascal and R. Dedonder, *Carbohydrate Res.*, 1972, **24**, 365.

Active immobilized forms of β-D-fructofuranosidase have been produced by entrapment in cellulose acetate and other fibres,[78, 79] by irradiation in the presence of acrylamide (to give a spongy polymeric matrix),[80] and by cross-linking with glutaraldehyde on magnetite.[81] The latter reaction produced a magnetic immobilized enzyme. A fibre-entrapped, mixed D-glucose oxidase, β-D-fructofuranosidase, and catalase immobilized system has also been produced.[79]

Fucosidases

The multiple forms of the α-L-fucosidase of human tissues have been reviewed, and their relations to known storage disorders involving carbohydrates discussed.[16] The enzymology (deficiency of α-L-fucosidase) and related chemical investigations of fucosidosis in man have been reviewed.[82]

The two forms of α-L-fucosidase from various human organs have been separated by gel filtration;[83] they differed in thermostability, pH stability and optima (4.5—5.0 and 5.5), and substrate specificities. However, both forms were active against 4-nitrophenyl β-D-glucopyranuronoside and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. Information on the presence of α-L-fucosidase in human amniotic fluid and placenta is of interest, since these sources can be used to study the enzyme in certain hereditary diseases. The activities towards 4-nitrophenyl α-L-fucopyranoside of α-L-fucosidase in the muscles of controls and patients with various muscular and neuromuscular diseases have been determined.[84] Increased activity of the enzyme was found only in severely deranged muscles resulting from multiple diseases.

Normal human-liver α-L-fucosidase has been separated into two forms by ion-exchange chromatography and by gel filtration.[85] The highly macromolecular form was adsorbed by DEAE-cellulose, whereas the lower-molecular-weight (5.0×10^4) form was not. Both forms (pH optima 5.0) possessed distinctive pH–activity profiles due to the relative thermal instability of the form of lower molecular weight. Human placental α-L-fucosidase has been purified by affinity chromatography on an agarose matrix to which L-fucosylamine had been coupled.[86] Polyacrylamide gel electrophoresis of the product gave two α-L-fucosidase bands. The enzyme was inactive against a number of glycosides, and, compared with the activity against 4-nitrophenyl α-L-fucopyranoside, that against 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside was only 3.7%.

Fairly strong α-L-fucosidase activity has been detected in human polymorphonuclear leucocytes.[87] With 4-nitrophenyl α-L-fucopyranoside as substrate, the enzyme (pH optimum 5.4—5.6, K_m 0.168 mmol l^{-1}) displayed a broad pH–activity

[78] W. Marconi, S. Gulinelli, and F. Morisi, *Biotechnol. Bioeng.*, 1974, **16**, 501.
[79] D. Dinelli, *Process Biochem.*, 1972, **7**, 9.
[80] K. Kawashima and K. Umeda, *Biotechnol. Bioeng.*, 1974, **16**, 609.
[81] E. Van Leemputten and M. Horisberger, *Biotechnol. Bioeng.*, 1974, **16**, 385.
[82] F. Van Hoof, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van ,Hoof, Academic Press, New York, p. 277.
[83] G. Y. Wiederschain, L. G. Kolibaba, and E. L. Rosenfeld, *Clinica Chim. Acta*, 1973, **46**, 305.
[84] N. C. Kar and C. M. Pearson, *Clinica Chim. Acta*, 1973, **45**, 269.
[85] D. Robinson and R. Thorpe, *Clinica Chim. Acta*, 1973, **47**, 403.
[86] J. A. Alhadeff, A. L. Miller, and J. S. O'Brien, *Analyt. Biochem.*, 1974, **60**, 424.
[87] J. L. Avila and J. Convit, *Biochim. Biophys. Acta*, 1974, **358**, 308.

profile. There was no clear evidence for the occurrence of several forms of the α-L-fucosidase, and, although the enzyme appeared to be thermally stable, it was extremely sensitive to thiol-blocking reagents. About 30% of the activity in cytoplasmic extracts was latent, but was unmasked by sonication, detergents, low osmotic pressures, and homogenization. The distribution pattern of the α-L-fucosidase in subcellular fractions of polymorphonuclear leucocytes is closely similar to that reported for other 'acid' glycoside hydrolases in the tissue. The results support the suggestion that α-L-fucosidase is associated with primary granules. The α-L-fucosidase activities in white blood cells from a female patient with fucosidosis and from her relatives have been studied; the enzyme was absent in the patient, whereas the value for the ratio of α-L-fucosidase : α-D-mannosidase activities for the parents was intermediate between those of the patient and the controls.[88]

Isoelectric focusing has been used to separate the β-D-fucosidase activity of porcine kidney from the other glycosidases present and to separate the multiple forms of α-L-fucosidase.[59] Comparative investigations showed that the β-D-fucosidase and β-D-galactosidase activities are exhibited by a single enzyme species.

β-D-Fucosidase activity has been found among the digestive enzymes in the gut of the polychaete *Neanthes virens*; the main sites of production of the extracellular enzyme were shown to be the oesophageal pouches and the interior parts of the intestine.[89]

α-L-Fucosidases obtained from the marine gastropods *Charonia lampas* and *Turbo cornutus* hydrolysed all the L-fucosidic linkages found in milk oligosaccharides.[90] The rates of hydrolysis of various linkages by the enzyme from *T. cornutus* were in the order α-L-fucosyl-(1 → 4)-(2-acetamido-2-deoxy-D-glucosyl) > α-L-fucosyl-(1 → 2)-D-galactosyl > α-L-fucosyl-(1 → 3)-(2-acetamido-2-deoxy-D-glucosyl), whereas the rates for the enzyme from *C. lampas* were in the order α-L-fucosyl-(1 → 2)-D-galactosyl > α-L-fucosyl-(1 → 4)-(2-acetamido-2-deoxy-D-glucosyl) > α-L-fucosyl-(1 → 3)-(2-acetamido-2-deoxy-D-glucosyl). The release of L-fucose from mouse myeloma immunoglobulin IgG glycopeptide by both enzymes demonstrated the wide range of specificities for substrates of different sizes and linkages. Aryl 1-thio-L-fucopyranosides have been tested as inhibitors of an α-L-fucosidase from a species of clam; 4-aminophenyl 1-thio-α-L-fucopyranoside showed competitive inhibition.[91]

The coleoptile cell wall of *Avena sativa* contains β-D-fucosidase.[92] It was suggested that indole-3-acetic acid facilitated the release of protons into the cell wall, thereby promoting the activities of the glycoside hydrolases, some of which may participate in cell-extension processes.

An α-L-fucosidase has been purified from *Bacillus fulminans* by fractional precipitation, gel filtration, and ion-exchange chromatography; L-fucose liberated

[88] I. Matsuda, S. Arashima, M. Anakura, and Y. Oka, *Clinica Chim. Acta*, 1973, **48**, 9.
[89] D. G. Kay, *Comp. Biochem. Physiol.*, 1974, **47A**, 573.
[90] M. Nishigaki, T. Muramatsu, A. Kobata, and K. Maeyama, *J. Biochem. (Japan)*, 1974, **75**, 509.
[91] M. L. Chawla and O. P. Bahl, *Carbohydrate Res.*, 1974, **32**, 25.
[92] K. D. Johnson, D. Daniels, M. J. Dowler, and D. L. Rayle, *Plant. Physiol.*, 1974, **53**, 224.

by the enzyme was determined by a microdiffusion method.[93] The enzyme (molecular weight 7—8 × 10^4) specifically hydrolysed terminal 2-*O*-α-L-fucopyranosyl-linkages in glycoproteins, glycopeptides, and oligosaccharides, but failed to cleave the α-L-fucopyranosyl linkage in each of 4-nitrophenyl α-L-fucopyranoside, lacto-*N*-fucopentaose II (containing a 1 → 4-linkage), and lacto-*N*-fucopentaose III (containing a 1 → 3-linkage). The apparent K_m values for desialylized porcine submaxillary mucin and the glycopeptide thereof, lacto-*N*-fucopentaose I, and 2′-fucosyl-lactose were 0.33, 1.6, 2.0, and 2.5 mmol l^{-1}, respectively.

Galactosidases

The multiple forms of α- and β-D-galactosidases of human tissues have been reviewed, and their relation to known storage disorders involving carbohydrate-containing molecules was discussed.[16] The abnormal enzymology of Fabry's disease (an α-D-galactosidase deficiency) has been considered in the light of recent experiments with highly purified enzymes obtained from human sources.[17] The structural, catalytic, and immunological properties of the enzymes involved were compared with their normal counterparts. The use of enzymes from humans in replacement therapy trials was encouraging. The enzymological and biochemical aspects of Fabry's disease,[94] G_{M_1}-gangliosidosis (an 'acid' β-D-galactosidase deficiency),[95] and Krabbe's disease or globoid cell leukodystrophy (a β-D-galactosidase deficiency) [96] have been reviewed, with particular emphasis on the activities of the enzymes involved towards glycolipid and ganglioside substrates.

A microscale assay for a β-D-galactosidase active against ganglioside G_{M_1} has been developed using as substrate G_{M_1} that had been tritiated exclusively in the non-reducing, terminal D-galactosyl residue.[97] The reaction was stimulated by anionic and cationic detergents, but was inhibited by neutral detergents. The enzyme present in human brain and liver was much more active towards 4-methylumbelliferyl β-D-galactopyranoside than towards G_{M_1}, and the enzyme from both organs was separated into two forms [molecular weights 6.0—7.0 × 10^4 (major), 6.0—8.0 × 10^5 (minor)] by gel filtration. The two forms isolated from liver by gel filtration corresponded to the two major forms found on starch gel electrophoresis; they were converted into electrophoretically slower forms on treatment with neuraminidase, suggesting that the two forms are glycoproteins. The mutation in G_{M_1} gangliosidosis appeared to result in severe reduction of the activities of both forms of β-D-galactosidase active towards ganglioside G_{M_1}.

A search has been made for new degradative pathways for D-galactosylceramides in an effort to explain the failure of these lipids to accumulate in the brains of children with Krabbe's disease.[98] Two new derivatives of D-galactosyl-

[93] N. Kochibe, *J. Biochem.* (*Japan*), 1973, **74**, 1141.
[94] J. A. Kint and D. Carton, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 357.
[95] F. Van Hoof, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 305.
[96] K. Suzuki and K. Suzuki, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 395.
[97] A. G. W. Norden and J. S. O'Brien, *Arch. Biochem. Biophys.*, 1973, **159**, 383.
[98] Y. N. Lin and N. S. Radin, *Lipids*, 1973, **8**, 732.

cerebroside were detected, and it was suggested that the aetiology of the disease does not involve the accumulation of D-galactosylceramides, with consequent formation of toxic products, but rather the malfunction of the corresponding galactoside hydrolase.

β-D-Galactosidase activities have been detected in human liver, placenta, urine, and blood platelets.[35] The α- and β-D-galactosidases of human liver have been separated by ion-exchange chromatography, which resolved the latter into three peaks. It was found that an activator is required for the hydrolysis of glycosphingolipids. The activator was partially purified, and it stimulated the hydrolyses of ganglioside G_{M_1} and trihexosylceramide by the α- and β-D-galactosidases, respectively. The requirement of a heat-stable activator to enable glycoside hydrolases to degrade the carbohydrate chains of glycosphingolipids suggests that the mechanism of catabolism is complex.

Human-liver 'acid' β-D-galactosidases A and B were bound by an immobilized form of concanavalin A.[99] Novel features for elution of the enzyme from the column were (i) an optimum in the concentration of the competitor sugar, and (ii) a marked dependence on temperature. Only 60% of the total 'acid' β-D-galactosidase was bound by immobilized wheat-germ agglutinin, whereas the L-fucose-binding protein from *Lotus tetraglobinus* showed no binding at all. The results suggested that the 'acid' β-D-galactosidases are glycoproteins containing α-D-mannopyranosyl and 2-acetamido-2-deoxy-D-glucosyl residues. 'Neutral' β-D-galactosidase did not bind to the concanavalin A derivative.

Each of the two forms of β-D-galactosidase isolated from human liver by gel filtration hydrolysed 4-methylumbelliferyl β-D-galactopyranoside and ganglioside G_{M_1}.[100] The conclusion that the two activities are promoted by the same enzyme species was based on the coincidence of the elution profiles on gel filtration, the simultaneous inactivation of both forms by heat and other treatments, the stabilization of both forms by Cl^- ions, and the effects of inhibitors. The isoenzymes were shown to differ in their molecular size, pH–activity profiles, resistance to dilution, and insensitivity to various inhibitors. The most significant difference between the two forms was in their saturation kinetics, which are consistent with the hypothesis that G_{M_1} acts as a modifier for one enzyme but not for the other. The membrane may serve a dual role in enzyme catalysis involving lipids: *viz.* as a medium where both the enzyme and the substrate are effectively concentrated, and as an activator of the enzyme by binding it to a specific membrane component.

Mixtures of chondroitin sulphate and human-liver β-D-galactosidases have an isoenzyme profile analogous to that found in Hunter and Hurler diseases; since the abnormality of the β-D-galactosidase isoenzyme and the reduction in enzymic activity can be neutralized with cetyl pyridinium chloride, the possibility of restoring the β-D-galactosidase activities in Hurler and Hunter diseases by the detergent has been investigated.[101] On adding the detergent to liver homogenates of patients with these diseases, a striking increase in β-D-galactosidase activity was observed, although the enzyme's activity was eliminated at very high

[99] A. G. W. Norden and J. S. O'Brien, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 193.
[100] M. W. Ho, P. Cheetham, and D. Robinson, *Biochem. J.*, 1973, **136**, 351.
[101] J. A. Kint, *Nature*, 1974, **250**, 424.

concentrations. The maximum activation obtained corresponded to only a partial restoration of the β-D-galactosidase activity; it appeared that cetyl pyridinium chloride dissociates the complex of glycosaminoglycan and enzyme with the restoration of enzymic activity, but that the detergent is also a strong inhibitor of uncomplexed β-D-galactosidase.

α-D-Galactosidase from human kidney has been separated into two components by isoelectric focusing and Cellogel electrophoresis.[102] One of the components (pI 4.3) was thermostable, whereas the other (pI 4.5) was thermolabile and sensitive to inhibition by *myo*-inositol. An α-D-galactosyl-D-galactosyl-D-glucosylceramidase activity was detected in both fractions, whose activities towards trihexosylceramide, melibiose, and 4-methylumbelliferyl α-D-galactopyranoside paralleled that towards 3-nitrophenyl α-D-galactopyranoside.

Incubation of antibodies to human-spleen α-D-galactosidase A with the homologous antigen significantly increased the thermal stability and the resistance to proteolysis of the enzyme.[103] Treatment of the enzyme with hexamethylene di-isocyanate (a bifunctional cross-linking reagent) furnished a derivative having higher stability and resistance to proteolysis than those of a derivative obtained using butyl isocyanate (a monofunctional reagent).

Three β-D-galactosidases in the small intestines of humans have been partially purified and characterized.[104] Quantitative methods of analysis applicable to single jejunal biopsies were then devised to study alterations of the enzymes in adults possessing low β-D-galactosidase activity and in congenital deficiencies involving β-D-galactosidase. The activity of brush-border β-D-galactosidase was reduced 10—20-fold in adults with specific, low β-D-galactosidase activity. The residual enzyme showed the same properties as that in adults with persistent high activity. In patients with congenital deficiencies of β-D-galactosidase, no brush-border activity was demonstrated, although other β-D-galactosidase activities are normal.

The activities of β-D-galactosidase (pH optimum 5.0, K_m 0.52 mmol l^{-1} towards 4-nitrophenyl β-D-galactopyranoside) have been determined in biopsy specimens of the rectal mucosa of cystinotic children and their parents, and controls.[39]

A sensitive, simple assay using a fluorogenic substrate has been developed to measure the α-D-galactosidase activity in plasma, sera, leucocytes, and urine.[105] A deficiency of α-D-galactosidase activity in these sources was shown to be diagnostic for hemizygotes and heterozygotes with Fabry's disease, which involves a deficiency of the specific α-D-galactosidase – α-D-galactosyl-D-galactosyl-D-glucosylceramidase. The pH optimum of the enzyme from each source was the same for heterozygotes and controls, and the K_m values were similar for hemizygotes, heterozygotes, and controls. The enzyme comprises heat-stable and -labile forms in all cases. It was concluded that the residual

[102] I. Kano and T. Yamakawa, *J. Biochem.* (*Japan*), 1974, **75**, 347.
[103] P. D. Snyder, F. Wold, R. W. Bernlohr, C. Dullum, R. J. Desnick, W. Krivit, and R. M. Condie, *Biochim. Biophys. Acta*, 1974, **350**, 432.
[104] N.-G. Asp and A. Dahlqvist, *Enzyme*, 1974, **18**, 84.
[105] R. J. Desnick, K. Y. Allen, S. J. Desnick, M. K. Raman, R. W. Bernlohr, and W. Krivit, *J. Lab. Clin. Med.*, 1973, **81**, 157.

activity in hemizygotes is a heat-stable form and that the α-D-galactosyl-D-galactosyl-D-glucosylceramidase is the heat-labile, *myo*-inositol-inhibited form.

β-D-Galactosidases have been purified from monkey livers and intestines, and the enzymes from both sources had broad pH–activity profiles towards 2-nitrophenyl β-D-galactopyranoside (pH optima 5.6 and 6.5—7.0, respectively).[106] Both enzymes were active towards synthetic glycosides, and, although inactive towards lactose, cellobiose, and phlorizin, the enzymes also hydrolysed synthetic β-D-glucopyranosides. Heavy-metal ions (Hg^{2+}, Ag^{+}, and Cu^{2+}) inhibited both β-D-galactosidases, indicating the presence of thiol groups.

β-D-Galactosidase from bovine testes has been purified by precipitation, fractionation with acetone, and affinity chromatography on a derivative of agarose coupled with 4-aminophenyl β-D-1-thiogalactopyranoside.[107] A wide variety of compounds containing terminal, non-reducing D-galactosyl residues, including proteoglycan fragments, glycoproteins, gangliosides, disaccharides, and synthetic D-galactosides, were hydrolysed by the enzyme (pH optimum 4.3); although the K_m values differed, the rate of reaction was the same for a number of the substrates. The enzyme (molecular weight 6.8×10^4) migrated as a single band on polyacrylamide gel electrophoresis, required thiol groups for activity, and exhibited transferase activity. Two distinct β-D-galactosidase activities were demonstrated in extracts of bovine liver; one was bound by the affinity column used for chromatography and was analogous to the testicular enzyme, whereas the other neither hydrolysed lactose nor transferred D-galactosyl residues from D-galactosides to D-glucose.

Ovine-brain β-D-galactosidase has been found to bind with concanavalin A to form an enzymically active precipitate.[57] The activity of this complex was inhibited by methyl α-D-glucopyranoside, which, depending on the pH of the medium, also dissociated the complex; maximum dissociation occurred at pH 4.0. Since the glycoside differentially dissociated the complexes formed with other enzymes and the phytohaemagglutinin, complex formation could be used to purify the β-D-galactosidase from a mixture of enzymes. The enzyme is assumed to be a glycoprotein.

The α- and β-D-galactosidase activities of porcine kidney have been separated from each other and other glycosidases present by isoelectric focusing.[59] Comparative investigation of the β-D-galactosidase and β-D-fucosidase activities of the preparation showed that they are manifest by a single enzyme species.

The localization of β-D-galactosidase activity in canine kidney has been investigated by a multiple-indicator dilution technique using [^{14}C]lactose as the substrate.[72]

The activities of rat-brain D-galactosylsphingosine (psychosine) and D-galactosylceramide (D-galactocerebroside) β-D-galactosidases have been compared.[108] The thermal stability of each enzyme was demonstrated, whereas a reference β-D-galactosidase (active towards 4-methylumbelliferyl β-D-galactopyranoside) was labile. Ion-exchange chromatography, isoelectric focusing (pI 4.45), and changes during development of the brain suggested that the two hydrolyses are

[106] B. Seetharam and A. N. Radhakrishnan, *Indian J. Biochem. Biophys.*, 1973, **10**, 3.
[107] J. J. Distler and G. W. Jourdian, *J. Biol. Chem.*, 1973, **248**, 6772.
[108] T. Miyatake and K. Suzuki, *J. Neurochem.*, 1974, **22**, 231.

catalysed by a single enzyme species. Glycolipids and gangliosides tritiated in the terminal, non-reducing D-galactosyl residues have been used in the determination of β-D-galactosidase in rat brain.[109] In all cases, the presence of taurocholate was required for activity, whereas all other detergents tested were ineffective. The apparent K_m values were considerably greater than those previously reported, and D-galactose was the only monosaccharide released. The assay procedure provides a method for determining β-D-galactosidases in tissues (including pathological specimens) and a reference system for testing the purification of specific glycolipid β-D-galactosidases. After the removal of phospholipids from rat-liver lysosomes, incubation of the lysosomes with phospholipase C did not affect the β-D-galactosidase activity, suggesting that phospholipids do not function in the manifestation of this lysosomal activity.[110]

Changes in the levels of β-D-galactosidase activity during development of chick embryos and intestines, and the effect of hydrocortisone on the enzyme's activity in the embryos, have been investigated.[75]

The α-D-galactosidase activity has been investigated in a study of the digestive enzymes in the midgut of *Stenotus binotatus*; the enzyme's distribution was compared with that in other heteropterous insects.[111] A similar study of the enzymes in the gut of the polychaete *Neanthes virens* has investigated the α- and β-D-galactosidase activities; the main sites for production of the enzymes were shown to be the oesophageal pouches and the interior parts of the intestine.

In a review of active-site-directed inhibitors and the mechanisms of action of glycoside hydrolases, the β-D-galactosidases of *Helix pomatia* and *Escherichia coli* have been considered in some detail.[14] Both α- and β-D-galactosidase activities have been found in a variety of marine invertebrates from the Sea of Japan, and the distribution of these enzymes in selected species of Coelenterata, Annelida, Arthropoda, Mollusca, Echinoderma, and Chordata was reported.[60]

The β-D-galactosidase activities of powders obtained from the termite *Trinervitermes trinervoides* have been investigated on storage.[112]

β-D-Galactosidases have been purified from almond emulsin and Jack-bean meal by ion-exchange chromatography and gel filtration; both enzymes specifically released D-galactose from immunoglobulin IgG glycopeptide.[113] The purified β-D-galactosidases did not act on lacto-*N*-fucopentaitols II and III, but hydrolysed the *O*-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-D-glucose)-linkage of lacto-*N*-neotetraitol much faster than the *O*-β-D-galactopyranosyl-(1 → 3)-(2-acetamido-2-deoxy-D-glucose)-linkage of lacto-*N*-tetraitol.

β-D-Galactosidase activity has been detected in the coleoptile cell wall of *Avena sativa*; it was suggested that indole-3-acetic acid enhances the release of protons into the cell wall, thereby promoting the activities of the cell-wall glycosidases, some of which may participate in cell-extension processes.[92] The β-D-galactosidase (pH optimum 4.5—5.5) was sensitive to Cu^{2+} ions and 4-chloromercuribenzoate, and its rate of turnover was shown to be exceptionally low. Enhancement of this activity by indole-3-acetic acid was prevented by

[109] T. Miyatake and K. Suzuki, *Biochim. Biophys. Acta*, 1974, **337**, 333.
[110] A. A. Pokrovsky, I. Y. Kon, and V. N. Soloviev, *Byull. Eksperim. Biol. i Med.*, 1974, **77**, 49.
[111] T. Takanona and K. Hori, *Comp. Biochem. Physiol.*, 1974, **47B**, 521.
[112] R. C. Potts and P. H. Hewitt, *Comp. Biochem. Physiol.*, 1974, **47B**, 317.
[113] M. Arakawa, S. Ogata, T. Muramatsu, and A. Kobata, *J. Biochem.* (*Japan*), 1974, **75**, 707.

abscisic acid and cycloheximide *in vivo*, and reduced by high buffer strengths and by low pH. The β-D-galactosidase of germinating seeds of *Canavalia ensiformis* has been purified by gel filtration and ion-exchange chromatography.[61]

Purification of the α-D-galactosidase from green coffee beans has been effected by affinity chromatography on bis(*N*-6-aminocaproyl) α-D-galactopyranosylamine coupled to an agarose derivative.[114] Polyacrylamide gel electrophoresis at pH 8.3 separated the purified enzyme into three active bands. Isolated cell walls of the callus of *Convolvulus arvensis* have been shown to contain α-D-galactosidase (pH optima 5.2—5.6, 6.0—6.4) and β-D-galactosidase (pH optimum 4.2—4.6). activities.[76] The β-D-galactosidase and other glycoside hydrolases of Jack-bean meal have been separated on an affinity-chromatography matrix of 4-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside coupled to an agarose derivative.[62]

The production of β-D-galactosidase by cultures of *Alternaria tenuis*, *Aspergillus awamori*, and *Escherichia coli* has been studied in detail.[115] A basic enzyme isolated from a *Coccobacillus* species by ion-exchange chromatography and gel filtration hydrolysed keratan sulphate, with the liberation of sulphated oligosaccharides and sulphated glycopeptides.[116] The enzyme, possibly an *endo*-β-D-galactosidase (pH optimum 5.5, temperature optimum 32 °C), was inhibited by heavy-metal ions, but by neither dithiothreitol nor H_4edta. The enzyme was assayed by measuring the turbidity produced by reaction of the residual keratan sulphate with hyamine. The rates of hydrolysis of different samples of keratan sulphate were compared, but other glycosaminoglycans and sulphated glycoproteins were unaffected.

2-(*R*)-Glyceryl β-D-galactopyranoside has been synthesized and tested as a substrate for the β-D-galactosidase of *Escherichia coli*.[117] The 2-(*S*)-isomer was also hydrolysed by the enzyme, which exhibited transglycosylation properties with lactose as a substrate and 1,2-*O*-isopropylideneglycerol as an acceptor. The effect of controlled feeding of glycerol on the production of β-D-galactosidase in batch cultures of a mutant of *E. coli*, constitutive for β-D-galactosidase, has been studied.[118] The occurrence of another β-D-galactosidase in *E. coli* has been demonstrated by comparison of the genetics of various strains of the organism.[119] Although this enzyme had virtually no activity towards lactose, it hydrolysed 2-nitrophenyl β-D-galactopyranoside, and its synthesis was induced by lactose.

Nucleophilic competition in reactions catalysed by β-D-galactosidase has been examined for the enzyme from *E. coli* towards 2-, 3-, and 4-nitrophenyl, phenyl, and 2- and 4-aminophenyl β-D-galactopyranosides and 2-nitrophenyl α-L-arabinopyranoside.[120] The rate-limiting step and individual rate constants were determined for each substrate; the rate of formation of the D-galactosyl-enzyme intermediate did not correlate with the Hammett values, suggesting that this process involves more than a single reaction. In testing the actions of various

[114] N. Harpaz, H. M. Flowers, and N. Sharon, *Biochim. Biophys. Acta*, 1974, **341**, 213.
[115] A. S. Tikhomirova, A. K. Kulikova, and R. V. Feniksova, *Microbiologia*, 1974, **43**, 257.
[116] S. Hirano and K. Meyer, *Connective Tissue Res.*, 1973, **2**, 1.
[117] T. J. Silhavy and W. Boos, *J. Biol. Chem.*, 1973, **248**, 6571.
[118] P. P. Gray, P. Dunnill, and M. D. Lilly, *Biotechnol. Bioeng.*, 1973, **15**, 1179.
[119] D. L. Hartl and B. G. Hall, *Nature*, 1974, **248**, 152.
[120] O M. Viratelle and J. M. Yon, *European J. Biochem.*, 1973, **33**, 110.

nucleophiles on the D-galactosyl-enzyme intermediates, a peculiar effect of 2-thiolethanol was noted. The quaternary structure of β-D-galactosidase, which consists of four identical subunits, has been studied by the isolation and characterization of mutants of *E. coli*.[121] Of numerous mutants examined, 19 were found to have enzymes with a reduced association of the subunits; these enzymes were dissociated into subunits at concentrations of urea that did not normally affect the enzyme. Mapping with overlapping deletions showed that the mutants affecting the quaternary structure of β-D-galactosidase are not distributed uniformly, but occur in five or six groups within the gene. Mapping also indicated that polypeptide sequences involved in the association of subunits and in the binding of substructures are contiguous. A model was proposed for β-D-galactosidase in which the binding sites of the substructure are provided by clefts between associated subunits.

Affinity chromatography has been used to determine whether incomplete polypeptide chains from a variety of *Z*-gene mutants are folded in the conformation necessary for recognition of the substrate by the β-D-galactosidase of *E. coli*.[122] Specific alkylation with a substrate analogue, *N*-bromoacetyl β-D-galactopyranosylamine, was also used to test for the presence of a binding site. The use of mutants containing different regions of the β-D-galactosidase allowed those portions of the molecule most directly involved in formation of binding sites to be determined. Contrary to the belief that active β-D-galactosidase is often formed in *rec*⁻ merozygotes containing a pair of mutations in the *Z* gene of the *lac* operon, with certain pairs of *E. coli* mutants it was noted that the enzyme did not yield two independent polypeptides, but only a single protomer.[123] Both genetic recombination and suppression have been ruled out as the source of this phenomenon, which was considered to be the result of the synthesis of one protein by two genes functioning independently. An immunoenzymic system for the study of immune responses *in vitro* is based on the production of antibodies directed towards different determinants of the β-D-galactosidase of *E. coli* in cultures of lymph-node fragments.[124]

Induction of β-D-galactosidases in *Fusarium oxysporum* and *Verticillium albo-atrum* grown with restricted supplies of a number of cell-wall carbohydrates has been investigated; in particular, the enzyme was induced by D-galacturonic acid and, to a lesser extent, by D-galactose.[125]

α-D-Galactosidase activity has been found in cultures of cell-free extracts of *Lactobacillus fermenti*, *L. brevis*, *L. buchneri*, *L. cellobiosis*, and *L. salivarius*.[126] The conditions under which enzymic activity was detected suggested that the enzyme is constitutive and is present in the soluble fraction of the cell. The enzyme preparations readily hydrolysed melibiose and other oligosaccharides containing (1 → 6)-linked α-D-galactopyranosyl residues at the terminal, non-reducing position. Although cell-free extracts of *L. fermenti* and *L. brevis* did not contain β-D-fructofuranosidase, they hydrolysed melibiose, raffinose, and

[121] J. Langridge, *Austral. J. Biol. Sci.*, 1974, **27**, 321.
[122] M. R. Villarejo and I. Zabin, *Nature New Biol.*, 1973, **242**, 50.
[123] B. N. Apte and D. Zipser, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2969.
[124] A. J. L. Marcario, E. Conway de Macario, and F. Celada, *Immunology*, 1973, **24**, 237.
[125] R. M. Cooper and R. K. S. Wood, *Nature*, 1973, **246**, 309.
[126] B. K. Mital, R. S. Shallenberger, and K. H. Steinkraus, *Appl. Microbiol.*, 1974, **26**, 783.

stachyose in decreasing order of activity. The enzymes exhibited maximal activity in the pH range 5.2—5.9 and the temperature range 38—42 °C, except for those from *L. fermenti* (40—44 °C) and *L. salivarius* (48—51 °C). The transglycosylase activities of the β-D-galactosidase from *L. cellobiosis* were also studied.

An active immobilized form of α-D-galactosidase has been prepared by cross-linking the enzyme on nylon.[127] Immobilized forms of β-D-galactosidase have been obtained by reaction of the enzyme with diazo-derivatives of porous glass,[128] with an alkylamine derivative of zirconia-coated porous glass,[129, 130] with stainless steel and other dense carriers coated with a layer of titanium oxide,[131] with glutaraldehyde cross-linking on porous titanic oxide,[130] and with aminoethylcellulose.[132] Entrapment in polyacrylamide gels by γ-irradiation, or by ammonium-persulphate-catalysed polymerization of acrylamide in the presence of the enzyme,[133] and also in fibres of cellulose acetate by a wet-spinning process [79] was also used to prepare immobilized forms.

Glucosidases

The purification of β-D-glucosidases by affinity chromatography on polysaccharide matrices has been described.[26] In one approach, such inhibitors as 4-aminobenzyl and 4-aminophenyl 1-thioglycopyranosides were used. Active-site-directed inhibitors and the mechanisms of the action of β-D-glucosidases from a number of sources have been reviewed.[14]

The multiple forms of α- and β-D-glucosidases present in human tissues have been reviewed, and their relation to storage disorders involving carbohydrate-containing molecules was discussed.[16] A review of Gaucher's disease (a deficiency of β-D-glucosidase that results in an accumulation of glucocerebroside) has covered the enzymic defect, the enzymological diagnosis, and the recognition of homo- and hetero-zygotes.[134] The abnormal enzymology of the disease has also been considered in the light of recent findings with purified enzymes from humans, and the structural, catalytic, and immunological properties of the enzyme were compared with those of the normal counterpart.[17] A review of type II glycogenesis has dealt with the purification, properties, and deficiency of lysosomal 'acid' α-D-glucosidase, with the role of the enzyme in the metabolism of glycogen, and with the hypersensitivity of other lysosomal enzymes.[135] The review also related the enzymology to the genetics, and discussed the detection of homo- and hetero-zygotes.

[127] J. H. Reynolds, *Biotechnol. Bioeng.*, 1974, **16**, 135.
[128] L. E. Wierzbicki, V. H. Edwards, and F. V. Koskowski, *Biotechnol. Bioeng.*, 1974, **16**, 397.
[129] H. H. Weetall, N. B. Havewala, W. H. Pitcher, C. C. Detar, W. P. Vann, and S. Yaverbaum, *Biotechnol. Bioeng.*, 1974, **16**, 295.
[130] H. H. Weetall, N. B. Havewala, W. H. Pitcher, C. C. Detar, W. P. Vann, and S. Yaverbaum, *Biotechnol. Bioeng.*, 1974, **16**, 689.
[131] F. X. Hasselberger, B. Allen, E. K. Paruchuri, M. Charles, and R. W. Coughlin, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1054.
[132] D. L. Regan, P. Dunnill, and M. D. Lilly, *Biotechnol. Bioeng.*, 1974, **16**, 333.
[133] P. S. Bunting and K. J. Laidler, *Canad. J. Biochem.*, 1973, **51**, 1598.
[134] R. O. Brady and F. M. King, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 381.
[135] H. G. Hers and T. de Barsy, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 197.

A search has been made for new degradative pathways for D-glucosylceramides in an effort to explain the failure of these glycolipids to accumulate in the brains of children with Gaucher's disease.[98] It was suggested that the aetiology of the disease does not involve an accumulation of D-glucosylceramide, with the consequent formation of toxic products, but rather a malfunction of the corresponding glycosidase. The activities of α-D-glucosidase (towards 4-nitrophenyl α-D-glucopyranoside) in the muscles of normals and of patients with various muscular and neuromuscular diseases have been determined.[84] Increased activities of the enzyme were found only in severely damaged muscles resulting from multiple diseases.

β-D-Glucocerebrosidase in human spleen is associated with a membrane-bound 'acid' β-D-glucosidase and could be separated from a soluble, 'neutral' β-D-glucosidase exhibiting no activity towards D-glucocerebroside.[136] Both the β-D-glucocerebrosidase and 'acid' β-D-glucosidase activities depended on an association of factor P (a heat-stable, soluble acid glycoprotein) with factor C (a heat-labile, membrane-bound protein). Solubilized and membrane-bound factor C could also be stimulated by sodium taurocholate to give both activities. The results were discussed in relation to Gaucher's disease and the clinical diagnosis thereof. The requirement for a third component in the association of factors C and P was predicted. The reconstitution of β-D-glucocerebrosidase from its macromolecular components depended on acid phospholipids, and the combination of factors C and P produced an active enzyme only in the presence of acid phospholipids.[137] Human-liver β-D-glucosidase was not adsorbed by immobilized forms of concanavalin A.[99]

Studies on the β-D-galactosidases of monkey livers and intestines have suggested that the β-D-glucosidase activities of the preparations are also manifest by the same enzyme species.[106]

β-D-Glucosidase from bovine spleen has been freed from other glycoside hydrolase activities by affinity chromatography on immobilized D-glucono-1,4-lactone.[138] β-D-Glucosyl-ceramidase and -sphingosidase activities (pH optima 4.5) were measured using 4-methylumbelliferyl β-D-glucopyranoside, β-D-glucopyranosyl-[1-^{14}C]ceramide, and β-D-glucopyranosyl-[1-^{14}C]sphingosine as substrates. Radiolabelled D-glucocerebroside was obtained when β-D-glucosidase from bovine spleen was incubated with 4-methylumbelliferyl β-D-glucopyranoside and [^{14}C]ceramide.[139] The radiolabelled product was also hydrolysed by the β-D-glucosidase, resulting in the liberation of [^{14}C]ceramide. Neither methyl β-D-glucopyranoside nor D-glucose was converted into D-glucocerebroside by this system.

An α-D-glucosidase has been purified from porcine liver by heat treatment, fractionation with acetone, gel filtration, ion-exchange chromatography, and isoelectric focusing; it was shown to be homogeneous on ultracentrifugation and polyacrylamide gel electrophoresis.[140] The 9.90*S* enzyme (pI 3.7) catalysed the

[136] M. W. Ho, *Biochem. J.*, 1973, **136**, 721.
[137] M. W. Ho and N. D. Light, *Biochem. J.*, 1973, **136**, 821.
[138] J. N. Kanfer, R. A. Mumford, S. S. Raghavan, and J. Byrd, *Analyt. Biochem.*, 1974, **60**, 200.
[139] S. S. Raghavan, R. A. Mumford, and J. N. Kanfer, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 99.
[140] K. Uchida and Y. Suzuki, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 195.

hydrolysis of various α-D-glucopyranosides and also the transfer of D-glycosyl units from such substrates to riboflavin (K_m 1.20 mmol l^{-1} with maltose). Amylose was hydrolysed almost completely to D-glucose by the enzyme, whereas maltotriose was obtained from maltose. The enzyme also catalysed the transfer of an α-D-glucosyl residue from maltose to pyridoxine, esculin, rutin, and adenosine, all of which appeared to be catalysed by a single enzyme.

The localization of α-D-glucosidase in canine kidneys has been studied by means of a multiple-indicator dilution technique with [^{14}C]maltose as a substrate.[72] The spatial relationship of the enzyme's location to receptors of the transport of D-glucose was also considered. A number of characteristics of the degradation of polysaccharides by the 'neutral' α-D-glucosidase from rabbit liver have been reported.[141]

The phloretin-β-D-glucosidase activity of hamster and rat small intestines follows the 'neutral' β-D-galactosidase activity during purification, but the two catalytic sites are partly independent.[142] The activity of the phloretin-β-D-glucosidase in baby rats is highest at birth, but it decreases to the level found in adults by the time of weaning. The removal of phospholipids from rat-liver lysosomes led to a sharp decrease in the activity of membrane-bound β-D-glucosidase.[111] Incubation of the lysosomes with phospholipase C also considerably decreased the activity of this enzyme, but incubation of the lipid-free lysosomes with non-ionic and anionic detergents partly restored the activity.

Changes in the levels of α-D-glucosidase activity in the intestines of chick embryos and chicks have been studied during development, and the effect of administration of hydrocortisone on the level in the embryo has been studied.[75]

In an investigation of the distribution of α- and β-D-glucosidases in the gut of the polychaete *Neanthes virens*, the main sites of production of the extracellular enzymes were shown to be the oesophageal pouches and the interior parts of the intestine.[89] The distributions of α- and β-D-glucosidases have been reported for selected species of Coelenterata, Annelida, Arthropoda, Mollusca, Echinoderma, and Chordata found in the Sea of Japan.[60]

The properties of the α-D-glucosidase activity located at the tip of the labellar chemosensory seta of the blowfly (*Phormia regina*) have been examined using an ultramicro method for determining the released hexose.[143] The enzymic activity was independent of pH over a wide range (pH 3—8) and was inhibited by tris buffer competitively and by low concentrations of Ca^{2+} ions. Comparison of these results with those for the α-D-glucosidase isoenzymes in an extract of the proboscis indicated that the intact enzyme has properties which distinguish it from soluble forms of the enzyme. Apparent similarities were observed between the electrophysiological properties of the intact enzyme and those of the labellar sugar receptor. The distributions of α-D-glucosidase in the salivary gland and midgut, and that of β-D-glucosidase in the midgut, of *Stenotus binotatus* have been compared with those of other heteropterous insects.[111] The stabilities of α- and β-D-glucosidases obtained from the termite *Trinervitermes trinervoides* to storage in powder form have been investigated.[112]

[141] I. S. Lukomskaya and N. A. Ushakova, *Doklady Akad. Nauk S.S.S.R.*, 1973, **210**, 960.
[142] V. Colombo, H. Lorenz-Meyer, and G. Semenza, *Biochim. Biophys. Acta*, 1973, **327**, 412.
[143] O. Koizumi, H. Kijima, and H. Morita, *J. Insect. Physiol.*, 1974, **20**, 925.

The reaction of almond β-D-glucosidase with 4-nitrophenyl β-D-glucopyranoside has been examined over the temperature range −45 to +25 °C in 50% aqueous DMSO.[144] Evidence was obtained at low temperatures for the existence of a D-glucosyl-enzyme intermediate, whose breakdown was rate-limiting; this provided a method for measuring the normality of the active site. At pH 5.9 and 25 °C, the mixed solvent caused a ten-fold increase in K_m (1.7 mmol l^{-1} = 0%), whereas V_{max} was unchanged; it appeared that DMSO was acting as a competitive inhibitor with K_i of 0.7 mol l^{-1}. Almond β-D-glucosidase catalysed the hydrolysis of 6-purinyl 1-thio-β-D-glucopyranoside to 6-thiopurine and D-glucose.[145] The enzyme-catalysed hydrolysis in the pH range 3.55—5.95 was characterized by a shallow, bell-shaped V_{max}–pH profile having a maximum at pH 5.3. The K_m value decreased with increasing pH, and the deuterium kinetic isotope effect ($V_{max\text{-}H_2O}/V_{max\text{-}D_2O}$) was unity (pH = pD = 5.35).

A β-D-glucoside of D-pantothenic acid was formed on incubation of the acid with such β-D-glucosyl donors as cellobiose, salicin, and 4-methylumbelliferyl, phenyl, and naphthyl β-D-glucopyranosides in the presence of almond β-D-glucosidase.[146] Almond β-D-glucosidase also exhibited β-D-galactosidase activity.[113]

The coleoptile cell wall of *Avena sativa* has been shown to contain β-D-glucosidase activity; activation of the enzyme *in vivo* appeared to be associated with indole-3-acetic acid.[92] Isolated cell walls of *Convolvulus arvensis* calluses contained α- and β-D-glucosidase activities (pH optima 4.6—5.0 and 5.2—5.6, respectively).[76]

The variation of solubility of the α-D-glucosidase of a bird-proof variety of *Sorghum vulgare* grain has been studied in alkaline solutions of sodium chloride.[147] A good correlation was found between the degree of insolubility of the enzyme and that of malt amylases. Although peptone was able to liberate the insoluble α-D-glucosidase in the presence of sodium chloride, the maximum effect was achieved by using a combination of peptone, urea, sulphite, and Triton. The insolubility of the enzyme was attributed to the formation of insoluble tannin–enzyme complexes.

Separation of the components of a cellulase complex from 'post breaker' tomato (*Lycopersicon esculentum*) has demonstrated the presence of a β-D-glucosidase (pH optimum 4.2).[148]

An *Actinoplanes* species has been found to possess a β-D-glucosidase activity that could be enhanced by the addition of methyl or phenyl β-D-glucopyranosides, gentiobiose, or salicin to growing cultures.[149] Addition of D-glucose, lactate, or acetate suppressed the activity to the constitutive level, but never below it. Both the constitutive and inducible enzymes were purified, and their properties were found to be identical (pH optimum 5.8—6.0). The heat stabilities were defined, and the enzymes were inactivated by Hg^{2+}, Cu^{2+}, Pb^{2+}, and Ag^{+} ions. Inhibition by 4-chloromercuribenzoate could be overcome by either L-cysteine or 2-thio-

[144] A. L. Fink and N. E. Good, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 126.
[145] L. R. Fedor and B. S. R. Murty, *J. Amer. Chem. Soc.*, 1973, **95**, 8410.
[146] F. Kawai, H. Yamada, and K. Ogata, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 831.
[147] T. G. Watson, K. H. Daiber, and L. Novellie, *Phytochemistry*, 1974, **13**, 901.
[148] F. E. Sobotka and D. A. Stelzig, *Plant Physiol.*, 1974, **53**, 759.
[149] C. J. Michalski and A. Domnas, *Carbohydrate Res.*, 1974, **33**, 153.

ethanol, indicating the presence of thiol groups. The enzyme (molecular weight 1.65×10^5, K_m 0.25 mmol l^{-1} with 4-nitrophenyl β-D-glucopyranoside) did not exhibit transferase activity.

An extracellular α-D-glucosidase (molecular weight 6.356×10^4) from *Aspergillus fumigatus* has been purified to homogeneity by polyacrylamide gel electrophoresis in sodium dodecyl sulphate and ultracentrifugation.[150] The enzyme (pH optimum 4.5, K_m 1.85 with maltose) readily hydrolysed maltose and isomaltose, but utilized αα-trehalose and 4-nitrophenyl α-D-glucopyranoside only very slowly. The enzyme comprises D-glucose (5.4), D-mannose (55.1), and 2-amino-2-deoxy-D-glucose (3.2 moles per mole); precipitation with trichloroacetic acid released all the D-glucosyl residues, whereas the other monosaccharides appeared to be bound covalently. Structural analyses were performed on the carbohydrate portion of the macromolecule, and alkaline degradation indicated the presence of 2-acetamido-2-deoxy-D-glucosyl-L-asparagine linkages. An extracellular β-D-glucosidase from *Aspergillus fumigatus* has been purified to homogeneity, as assessed by physicochemical methods.[151] Specificity studies indicated that the enzyme (molecular weight 4.08×10^4, pH optimum 5.0) has a preference for D-glucosyl residues in (1 → 2)- and (1 → 3)-linkage. The purified enzyme contains D-glucose (33), D-mannose (17), and 2-amino-2-deoxy-D-glucose (2 moles per mole), but the D-glucosyl residues do not appear to be covalently bound. Proteolysis yielded two glycopeptides, suggesting that there are two carbohydrate chains per molecule, and other evidence suggested that the glycopeptide linkage is of the 2-acetamido-2-deoxy-D-glucosyl-L-asparagine type.

Growth of *Fomes annosus* on cellobiose produced more β-D-glucosidase than growth on D-glucose.[152] The enzyme was separated into four bands (pI's 5.4, 5.9, 6.4, and 6.7) by isoelectric focusing, and various features of this spectrum for both conditions of growth were studied. The production of β-D-glucosidase and cellulase activities by *Mucor pusillus* and *M. miehei* cultured on wheat-bran suspensions has been investigated.[153]

The α-D-glucosidase of *Saccharomyces cerevisiae* has been used to investigate the active-site-directed inhibitors and the mechanisms of action of glycoside hydrolases.[14] The synergistic effect of the β-D-glucosidase component of a cellulase from *Trichoderma koningii* with other components of the complex has been investigated.[154]

Active immobilized forms of β-D-glucosidase have been obtained by attachment of the enzyme to glass directly or by way of titanium chelates.[155]

Glucuronidases

In a study of the activities of β-D-glucuronidase in normal and atherosclerotic human aortas, it was shown that the levels of activity could be related to various stages of the disease.[33]

[150] M. J. Rudick and A. D. Elbein, *Arch. Biochem. Biophys.*, 1974, **161**, 281.
[151] M. J. Rudick and A. D. Elbein, *J. Biol. Chem.*, 1973, **248**, 6506.
[152] A. Hüttermann and C. Volger, *Arch. Mikrobiol.*, 1973, **93**, 195.
[153] G. A. Somkuti, *J. Gen. Microbiol.*, 1974, **81**, 1.
[154] G. Halliwell and M. Griffin, *Biochem. J.*, 1973, **135**, 587.
[155] J. F. Kennedy and P. M. Watts, *Carbohydrate Res.*, 1974, **32**, 155.

β-D-Glucuronidase has been isolated from human liver, placenta, platelets, and urine by affinity chromatography on a heterologous antibody attached to a modified agarose.[35] The levels of activity of the enzyme present in these sources were measured. Fibroblasts deficient in the enzyme, and in which glycosaminoglycans accumulated, could be corrected in culture by the enzymes from the above sources, although the enzyme from blood platelets was most effective.

The activities of β-D-glucuronidase (pH optimum 4.0, K_m 0.70 mmol l^{-1} towards 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside) in biopsy specimens of rectal mucosae of cystinotic children and their parents and controls have been measured; the activities correlated with other glycoside hydrolase activities of the control group.[39]

Although human saliva contains a β-D-glucuronidase, it also contains an inhibitor of the enzyme.[156] The inhibitor (molecular weight 3.8×10^5) contains hexose (16.6), fucose (4.9), 2-amino-2-deoxyhexose (15.7), and sialic acid (12.8%).

The urinary β-D-glucuronidase activity of children with protein malnutrition (kwashiorkor) has been shown to be significantly higher than that of controls, but the level of activity decreased on recovery.[157] There was no correlation between the activities of the enzyme in the sera and urine of the patients.

Ovine-brain β-D-glucuronidase has been found to bind with concanavalin A to form an enzymically active precipitate, indicating that the enzyme is a glycoprotein.[57] The activity of the complex was inhibited by methyl α-D-glucopyranoside, which also dissociated the complex (maximum dissociation at pH 4.0). Complex formation was used to purify the β-D-glucuronidase from a mixture of other glycoside hydrolases.

In a study of the selective release of lysosomal glycoside hydrolases from mice macrophages by immune complexes, time- and dose-dependent release of β-D-glucuronidase were demonstrated.[158] The interactions of the β-D-glucuronidase from rat preputial glands with substrates containing modified carboxy-groups have been investigated.[159] Methyl esters and amides of 4-nitrophenyl β-D-gluco- and -galacto-pyranuronosides had no inhibitory effect on the hydrolysis of 4-nitrophenyl β-D-glucopyranoside, and it was concluded that the carboxy-group is an essential structural feature of the subsite utilized in the formation of the enzyme–substrate complex.

The distribution of β-D-glucuronidase activity in the gut of the polychaete *Neanthes virens* has been defined; the main sites for the production of the enzyme are the oesophageal pouches and the interior parts of the intestine.[89] β-D-Glucuronidase activity has been found in the digestive systems of 18 species of Echinoderms, but the distribution of the enzyme was unrelated to the feeding habits of the animals.[160]

Total homogenates of early stages of embryogenesis, of larvae in different instars, of pharate pupae and adults, and of freshly emerged adults of the blowfly (*Crysomyia rufiferacis*) contained part of the β-D-glucuronidase bound to RNA.[161]

[156] W. Sakamoto, O. Nishikaze, and E. Sakakibara, *J. Biochem.* (*Japan*), 1974, **75**, 675.
[157] A. Begum, *Clinica Chim. Acta*, 1973, **46**, 229.
[158] C. J. Cardella, P. Davies, and A. C. Allison, *Nature*, 1974, **247**, 46.
[159] V. A. Nesmeyanov, S. E. Zurabyan, I. E. Sosinskaya, and A. Y. Khorlin, *Biokhimiya*, 1974, **39**, 141.
[160] D. Cornet and M. Jangoux, *Comp. Biochem. Physiol.*, 1974, **47B**, 45.
[161] A. T. Varute and V. A. Sawant, *Insect Biochem.*, 1972, **2**, 371.

The bound form is largely microsomal in origin, whereas the lysosomal β-D-glucuronidase showed no binding with RNA. The activity levels of the RNA-bound enzyme were measured at various stages of embryogenesis, larval growth, and metamorphosis; digestion with ribonuclease affected some of the properties of the enzyme, including a change in the pH optimum from 5.2—5.4 to 4.2—4.4 and the induction of thermal stability. Alterations in the bound enzyme during the development of the blowfly were examined in relation to the free β-D-glucuronidase present.

Iduronidases

The multiple forms of α-L-iduronidase present in human tissues have been reviewed, and their relation to carbohydrate-storage disorders was discussed.[16]

Interference by protein in assays of α-L-iduronidase with a phenyl α-L-idopyranuronoside can be avoided by measuring the phenol released with the Folin–Ciocalteau carbonate reagent.[162] The measurement is based on the reaction of *N*,2,6-trichloro-4-benzoquinoneimine with phenol to form indolylphenol, which forms a blue chromophore (λ_{max} 610 nm) when the solution is made alkaline.

Mannosidases

The general properties of α-D-mannosidases, the effect of Zn^{2+} ions on the enzymes *in vivo*, and the action of the enzymes on naturally occurring substrates (including macromolecules) have been reviewed.[163]

The multiple forms of α-D-mannosidase occurring in human tissues have been reviewed.[16] Mannosidosis has been discussed with respect to the deficiency of α-D-mannosidase and the enzymology and diagnosis of the disease.[164]

The activities of α-D-mannosidase in the muscles of patients with various muscular and neuromuscular diseases have been determined.[84] Increased activity was found only in deranged muscles resulting from multiple diseases. The levels of α-D-mannosidase activity in human liver, placenta, blood platelets, and urine have been measured.[35]

The α-D-mannosidase (pH optimum 4.3) present in human-blood leucocytes was shown to be somewhat unstable, and K_m and V_{max} values were higher for acetate buffer than for citrate buffer.[165] D-Mannono-1,5-lactone inhibited the enzyme, which was inactivated by Ag^+, Hg^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, and Mn^{2+} ions; inactivation could be reversed by H_4edta and was either reversed or prevented by Zn^{2+} ions. It was proposed that the α-D-mannosidase is a dissociable Zn^{2+}–protein complex in which Zn^{2+} ions are essential for enzymic activity. The subcellular distribution and structure-linked latency of the enzyme are compatible with its localization mainly in primary granules or lysosomes.

The α-D-mannosidase activity in the white blood cells of a patient with fucosidosis was increased, and the enzyme was relatively thermostable.[88] The

[162] J. Singh, P. Niebes, and N. Di Ferrante, *F.E.B.S. Letters*, 1974, **45**, 248.
[163] S. M. Snaith and G. A. Levvy, *Adv. Carbohydrate Chem. Biochem.*, 1973, **28**, 401.
[164] P. A. Öckerman, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. Van Hoof, Academic Press, New York, 1973, p. 291.
[165] J. L. Avila and J. Convit, *Clinica Chim. Acta*, 1973, **47**, 335.

apparent K_m values were similar for the patient and controls, and the ratios of α-L-fucosidase to α-D-mannosidase activities for the patient's parents were intermediate between those of the patient and the controls. The α-D-mannosidase activity in sera and leucocytes from patients with mannosidosis and from their parents has been measured at pH values of 4.4 and 6.0.[50] The disease could be diagnosed if the results were expressed as either the total or the specific activity at pH 4.4, or as the ratio of the enzymic activity at the two pH values, but the heterozygotes could not be distinguished from controls. However, the three groups were recognized by relating the 'acid' α-D-mannosidase activity to the total β-D-acetamidodeoxyglucosidase activity. The characteristics of the enzyme from each of the three groups differed on ion-exchange chromatography.

The α-D-mannosidase activity of human cerebrospinal fluid has been detected by a spectrofluorometric assay, which also permitted a correlative study with the β-D-acetamidodeoxyglucosidase activity to be made.[51]

α-D-Mannosidase has been found as a component of the Golgi membranes of rat liver; deoxycholate was required to release the enzyme from the membrane.[166] In addition to its distinctive subcellular locale, the Golgi enzyme could be distinguished from lysosomal and soluble, cytoplasmic α-D-mannosidases by its pH optimum, electrophoretic behaviour on polyacrylamide gel, rate of thermal inactivation, and kinetic properties.

The distribution of α-D-mannosidase in the gut of the polychaete *Neanthes virens* has been determined; the main sites for the production of the extracellular enzyme were shown to be the oesophageal pouches and the interior parts of the intestine.[89] Both α- and β-D-mannosidase activities have been found in a variety of invertebrates from the Sea of Japan; the distribution of the enzymes in selected species of Coelenterata, Annelida, Arthropoda, Mollusca, Echinoderma, and Chordata was examined.[60]

The coleoptile cell wall of *Avena sativa* contains α-D-mannosidase, and it was suggested that indole-3-acetic acid facilitated the release of protons into the cell wall, thereby promoting the activities of the glycoside hydrolases.[92] The α-D-mannosidase from germinating seeds of *Canavalia ensiformis* has been purified by gel filtration.[61]

Isolated cell walls of the callus of *Convolvulus arvensis* have been shown to contain α- and β-D-mannosidase activities (pH optima 4.4—5.0 and 4.7—6.2, respectively).[76] The α-D-mannosidase of Jack-bean meal has been separated from other glycoside hydrolases by affinity chromatography on a derivative of agarose coupled to 4-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside.[62]

Sialidases

The effects of neuraminidase on the development and growth of tumours have been discussed.[167, 168]

Membrane-bound neuraminidase has been purified from calf brains, and the pH optimum of the purified enzyme was found to vary from pH 3.1 to 4.0

[166] B. Dewald and O. Touster, *J. Biol. Chem.*, 1973, **248**, 7223.
[167] B. H. Sandford, *J. Nat. Cancer Inst.*, 1973, **51**, 1393.
[168] L. Weiss, *J. Nat. Cancer Inst.*, 1973, **51**, 1394.

according to the substrate used.[169] The enzyme had the highest affinity and V_{max} for gangliosides. It was inhibited by an excess of the substrate only in the case of gangliosides, for which the maximum rate of hydrolysis occurred at 70 °C. Although unaffected by Na^+ and Li^+ ions, the enzymic activity was enhanced by K^+ ions and was inhibited by NH_4^+ ions. Bivalent cations also inhibited the enzyme, but anions had no demonstrable effect. The maximum rate of hydrolysis of the disialoganglioside GD_1a was reached at a substrate concentration close to the critical micellar concentration.[170] The kinetics were maintained in the presence of deoxycholate, cholate, dodecylsulphate, and Triton X-100, and the effects of other detergents were also examined. Anti-neuraminidase activity was detected in the sera and in nasal secretions of cattle in a study of immunity against parainfluenza-3 virus.[171]

In a study of normal and transformed hamster cells in tissue cultures, sialidase activity towards extracellular gangliosides was found in all the transformed cell lines examined, whereas it was not detected in untransformed cells.[172] The relation between sialidase activity and the transformation of cells was discussed.

Changes in the soluble and particulate neuraminidases of rat brain and liver during development have been investigated.[173]

Plasma membranes and lysosomes isolated from rat liver have been assayed for sialidase activity; the enzymes from the two sources differed in substrate specificities, stability to heat, inhibition by Cu^{2+} ions, K_m values, and dependence on pH.[174] Hepatoma plasma membranes also exhibited sialidase activity.

Three partially purified neuraminidases from toad (*Bufo arenarum*) oviduct differed in their pH-dependence, kinetics, stability, and inactivation by 2-thioethanol.[175]

Contrary to previous reports, a matrix prepared by coupling *N*-(4-aminophenyl)oxamic acid to a derivative of agarose has been found to be unsatisfactory for the isolation of neuraminidase from *Clostridium welchii* by affinity chromatography.[176] Non-specific adsorption of haemagglutinins, haemolysin, and phospholipase C also occurred, and it appeared that the matrix acts as an ion-exchanger. A single-step, ion-exchange process has been developed to obtain *C. perfringens* neuraminidase free from proteolytic enzymes.[177] The stability of the enzyme to heat was investigated.

Glycoproteins and oligosaccharides containing terminal 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid units have been compared as substrates for a neuraminidase from *Myxovirus influenzae*.[178] Purified A_2/1957

[169] A. Preti, A. Lombardo, B. Cestaro, S. Zambotti, and G. Tettamanti, *Biochim. Biophys. Acta*, 1974, **350**, 406.
[170] G. Tettamanti, B. Cestaro, A. Lombardo, A. Preti, B. Venerando, and V. Zambotti, *Biochim. Biophys. Acta*, 1974, **350**, 415.
[171] B. Morein, S. Höglund, and R. Bergman, *Infection and Immunity*, 1973, **8**, 650.
[172] C.-L. Schengrund, R. N. Lausch, and A. Rosenberg, *J. Biol. Chem.*, 1973, **248**, 4424.
[173] R. Carubelli and D. R. P. Tulsiani, in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, 1972, p. 509.
[174] A. Visser and P. Emmelot, *J. Membrane Biol.*, 1973, **14**, 73.
[175] N. R. De Martínez and J. M. Olavarria, *Biochim. Biophys. Acta*, 1973, **320**, 301.
[176] J. I. Rood and R. G. Wilkinson, *Biochim. Biophys. Acta*, 1974, **334**, 168.
[177] M. W. C. Hatton and E. Regoeczi, *Biochim. Biophys. Acta*, 1973, **327**, 114.
[178] C. Bottex, G. Chatot, and R. Fontanges, *Compt. rend. Soc. Biol.*, 1974, **167**, 1837.

influenza neuraminidase and subunits thereof have been examined by isoelectric focusing in sugar gradients.[179] The native enzyme comprises a number of active components (pI's 5.2, 5.4, 5.5, 5.8, 6.2, and 6.5), two of which predominate. When the thiol groups of the subunits were blocked with iodo-[^{14}C]acetamide, 80% of the isotope incorporated was located in a single subunit (molecular weight 5.1×10^4). Other results suggested that the pI of the native enzyme is 1.5—2.0 units higher than that of the subunits. It was concluded that the carboxy-groups of the side-chains are masked in the native enzyme, and that isoelectric heterogeneity may result from conformation-dependent variations arising from the acid–base dissociation of these groups.

A thousand micro-organisms have been screened for the induction of neuraminidase activity, using colominic acid as the sole source of carbon in the culture media.[180] Neuraminidases with high activity and wide specificities were obtained from culture filtrates of *Penicillium urticae*, *Sporotrichum shenckii*, a *Streptomyces* species, and an unidentified bacterium 63831–1. The neuraminidases were purified by chromatography on an agarose derivative to which *N*-(4-aminophenyl)oxamic acid had been coupled. The purified enzymes liberated 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid from substrates in which the residue is either α-(2 → 8)-, α-(2 → 6)-, or α-(2 → 3)-linked.

The action of *Vibrio cholerae* neuraminidase on the surface of intact cells and isolated sialolipids therefrom has been studied.[181] Neuraminidase has been found in a strain of *Vibrio* species isolated from a patient with diarrhoea.[182] The thermolabile enzyme (pH optimum 5), which resembles the neuraminidase of *V. cholerae*, was activated by Ca^{2+} ions.

Xylosidases

Both α- and β-D-xylosidase activities have been found in invertebrates from the Sea of Japan; the distribution of the enzyme in selected species of Coelenterata, Annelida, Arthropoda, Mollusca, Echinoderma, and Chordata was investigated.[60]

β-D-Xylosidase activity has been detected in the gut of the polychaete *Neanthes virens*; the main sites for the production of the enzyme are the oesophageal pouch and the interior parts of the intestine.[89]

The coleoptile cell wall of *Avena sativa* has been found to contain a β-D-xylosidase, and it was proposed that the enzyme's activity *in vivo* is enhanced by the release of protons induced by indole-3-acetic acid.[92]

Of numerous organisms tested for their ability to produce β-D-xylosidases, the best results were obtained with *Aspergillus niger* and *Penicillium wortmanni*; the effects of inducers and surfactants on the yields of the enzyme were examined with these organisms.[183] In most instances, the enzymic activities far exceeded those reported for other sources. The β-D-xylosidases hydrolysed aryl and alkyl β-D-xylopyranosides, oligomers of D-xylose, and β-D-xylopyranosyl-L-serine, but neither β-D-xylosyldextran nor α-D-xylosides.

[179] A. P. Kendal, M. P. Kiley, and E. A. Eckert, *Biochim. Biophys. Acta*, 1973, **317**, 28.
[180] Y. Uchida, Y. Tsudada, and T. Sugimori, *Biochim. Biophys. Acta*, 1974, **350**, 425.
[181] N. W. Barton and A. Rosenberg, *J. Biol. Chem.*, 1973, **248**, 7353.
[182] H. E. Müller, *Infection and Immunity*, 1973, **8**, 430.
[183] E. T. Reese, A. Maguire, and F. W. Parrish, *Canad. J. Microbiol.*, 1973, **19**, 1065.

endo-D-Acetamidodeoxyglucosidases

A crude preparation of β-amylase from barley was found to contain lytic principles active against bacterial cell walls; isoelectric focusing yielded a component (pI 6.8) which appeared to be an *endo*-D-acetamidodeoxyglucosidase.[184] The enzyme showed limited activity against intact cell walls, but hydrolysed the glycans of bacterial cell walls from which non-peptidoglycan components had been partly removed. Even after repeated isoelectric focusing, the *endo*-D-acetamidodeoxyglucosidase retained β-amylase activity.[185] Determinations were made for the enzyme of (i) the dependence on pH, (ii) the susceptibility to ionic strength, metal ions, and thiol and chelating reagents, (iii) the stability to heat and preservatives, and (iv) the affinity for substrates.

Agarases

An extracellular agarase was produced when *Cytophaga flevensis*, a new agarolytic flexibacterium, was grown on either agar or melibiose.[186] The enzyme was shown to be highly specific for polysaccharides containing neoagarobiose units, and it also hydrolysed neoagaro-oligosaccharides with a degree of polymerization greater than four.

Alginate Lyases

Alginate lyase present in the hepatopancreas of a mollusc (*Littorina* species) has been purified to homogeneity by ion-exchange chromatography and polyacrylamide gel electrophoresis.[187] The enzyme (molecular weight 4.0×10^4, pH optimum 5.6) was stable in the pH range 4—8, but was inactivated on heating for 1 h at 50 °C. The transeliminase action of the enzyme operated preferentially between contiguous D-mannuronic acid residues in alginic acid.

α-Amylases

An automated method for the determination of α-amylase activity in sera and urine has used a water-insoluble, dyed (Cibachronblue F 36-A) starch as substrate.[188]

In a study of the purification of amylases by affinity chromatography, various types of ligand have been attached to the polysaccharide matrix.[26]

α-Amylase has been isolated from human submandibular saliva by gel filtration, precipitation with ammonium sulphate, and ion-exchange chromatography.[189] The purified enzyme was separated by gel filtration into families of isoenzymes, which were compared with those from human-parotid α-amylase. The enzymes from the two sources possess identical specific activities, isoenzyme patterns (polyacrylamide gel electrophoresis), and proportions (25%) of carbohydrate. Whereas the B forms contain no carbohydrate, the A forms from

[184] S. Iwata, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 171.
[185] S. Iwata, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 179.
[186] H. J. van der Meulen, W. Harder, and H. Veldkamp, *J. Microbiol. Serol.*, 1974, **40**, 329.
[187] L. A. Elyakova and V. V. Favorov, *Biochim. Biophys. Acta*, 1974, **358**, 341.
[188] A. Mazzuchin, C. Weggel, and C. J. Porter, *Clinical Chem.*, 1973, **19**, 1187.
[189] D. L. Kauffman, S. Watanabe, J. R. Evans, and P. J. Keller, *Arch. Oral Biol.*, 1973, **18**, 1105.

saliva and parotid contain 6 and 7 moles of carbohydrate, respectively. Isoelectric focusing of submandibular salivary α-amylase gave four isoenzyme bands [1A (18%), 1B (10%), 2A (9%), 2B (63%); pI's 5.9—6.4].[190] Although the amino-acid compositions are the same, positional differences were noted for certain residues. Differences between the 1A and 1B forms (molecular weights 5.7×10^4) and the 2A and 2B forms (molecular weights 5.4×10^4) were attributed to the presence of bound carbohydrate in the former. The enzymes contain D-galactose, D-mannose, L-fucose, and 2-amino-2-deoxy-D-glucose (2 : 3 : 3 : 3), and the 1A form also contains 2 moles of *N*-acetylneuraminic acid.

Six hexaploid albumins present in wheat kernels inhibited the α-amylases from human saliva, chick pancreas, yellow mealworm (*Tenebrio molitor*), *Aspergillus oryzae*, and *Bacillus subtilis*.[191] The inhibitors were characterized according to their molecular weights, c.d. and fluorescence spectra, amino-acid compositions, and specificities. The inhibitory activities of the proteins, several of which appeared to be related, were investigated.

In a simple method for screening human sera for macroamylasemia, the samples were chromatographed on thin-layer plates of Sephadex G-200 that had been pre-equilibrated with a buffer containing starch.[192] After incubation, the plates were sprayed with a solution of iodine to reveal α-amylase and macroamylase as well-defined spots.

The normal levels of urinary excretion of α-amylase for humans have been determined by an amylochrome assay.[193]

Porcine pancreatic α-amylase has been shown to contain two thiol groups and one tightly-bound Ca^{2+} ion per molecule.[194] Derivatization of the enzyme with either 5,5′-dithio-bis-(2-nitrobenzoic acid), iodoacetamidonaphthol, or Hg^{2+} ions did not delete its activity, indicating that the thiol groups are not required for activity. The pH–activity profiles of the derivatized enzyme were normal, but the specific activities were depressed. Certain of the thiol groups could be derivatized only after removal of calcium ions, and it was concluded that two of the thiol groups lie close together. Another study on the masking of α-amylase with 5,5′-dithio-bis-(2-nitrobenzoic acid) in the presence of H_4edta has reported that demasking is a pseudo-first-order process, whereas the masking process is first-order.[195] Part of the amylase was found in the —S—S— form, but complete blocking abolished the activity. One of the thiol groups does not appear to be present at the catalytic site, and inhibition of the masking reaction by a substrate analogue (maltotriose) suggested that the thiol groups are part of, or close to, the binding site(s).

Canine pancreatic α-amylase has been purified: under optimal conditions for assay of the enzyme with Phadebas starch as substrate, the addition of albumin increased the activity to different extents at different pH values.[196] Activation was greatest in the acid region. A similar variation in the activity with pH was

[190] J. W. Mayo and D. M. Carlson, *Arch. Biochem. Biophys.*, 1974, **163**, 498.
[191] V. Silano, F. Pocchiari, and D. D. Kasarda, *Biochim. Biophys. Acta*, 1973, **317**, 139.
[192] T. L. Peeters and G. R. Vantrappen, *Clinica Chim. Acta*, 1973, **47**, 437.
[193] B. Klein and J. A. Foreman, *Clinical Chem.*, 1973, **19**, 1226.
[194] M. L. Steer, N. Tal, and A. Levitzki, *Biochim. Biophys. Acta*, 1974, **334**, 389.
[195] G. Pommier, P. Cozonne, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1974, **350**, 71.
[196] M. D. O'Donnell and K. F. McGeeney, *Enzyme*, 1974, **18**, 356.

shown by the addition of either sodium taurodeoxycholate, Triton X-100, or polyethylene glycol. Both albumin and taurodeoxycholate produced an increase in V_{max}, while albumin also caused a decrease in K_m.

A micro-modification of the 3,5-dinitrosalicylic acid method has been used for measuring the secretion of amylase from fragments of mouse pancreas.[197] The amylase activity was used to monitor the secretion of exocrin, and the effects of hypoxin and 2,4-dinitrophenol on the secretion of amylase were also investigated. The output of amylase from superfused pancreatic tissue has been monitored by an automated procedure based on the liberation of dialysable, fluorogenic products from amylopectin anthranilate.[198] Stimulation of the enzyme with pancreozymin and acetylcholine was investigated using this extremely sensitive method.

In a study of the secretion of amylase from rat parotid gland, evidence was obtained for the participation of a protein kinase that depends on adenosine 3′,5′-cyclic phosphate for activity.[199] The effects of adrenalin, glucagon, insulin, and dibutyryl adenosine 3′,5′-cyclic phosphate on the α-amylase activities in rat liver and sera have been investigated.[200]

Amylase is one of the digestive enzymes found in the salivary gland and midgut of the bug *Stenotus binotatus*, and the distribution of the enzyme has been compared with that in other heteropterous insects.[111]

A quantitative determination of α-amylases in cereals and products derived therefrom has been based on colorimetric measurement of the components released from Phadebas starch (a cross-linked, dyed starch).[201] The technique is simple to operate and gave excellent correlations with viscosimetric and iodine-based methods; there is no interference by β-amylase. A brief review of the induction of gibberellic acid and α-amylase by germinating cereal seeds has described the properties of various isoenzymes.[202]

Electrophoresis of amylases from the aleurone layers of barley (*Hordeum vulgare*) seeds yielded seven isoenzymes (each of molecular weight 4.3×10^4), which were identified as α-amylases.[203] Gel filtration of the original enzyme gave seven fractions (molecular weights $0.3—4.5 \times 10^4$); the six of lowest molecular weight showed little or no activity, whereas the component of highest molecular weight (4.5×10^4) gave the seven isoenzyme bands on electrophoresis. These isoenzymes were thermally labile and were not stabilized by the presence of the substrate or a protease inhibitor (phenylmethylsulphonyl fluoride). The polymorphism of the α-amylase prepared from barley seeds has been studied by immunoelectrophoresis and immunoadsorption in a gel containing immune antiserum to malt-barley α-amylase.[204] The enzyme from germinated seeds was antigenically heterogeneous, and the two antigens were demonstrated to arise by different routes during germination. Most of the enzyme activity extracted from

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[198] E. K. Matthews, O. H. Petersen, and J. A. Williams, *Analyt. Biochem.*, 1974, **58**, 155.
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[200] K. Hammerton and M. Messer, *Clinica Chim. Acta*, 1973, **47**, 283.
[201] W. C. Barnes and A. B. Blakeney, *Stärke*, 1974, **26**, 193.
[202] T. Akazawa, in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, 1972, p. 449.
[203] D. E. Bilderback, *Plant Physiol.*, 1974, **53**, 480.
[204] J. Daussant, A. Skakoun, and M. L. Niku-Paavola, *J. Inst. Brewing*, 1974, **80**, 55.

the seeds at various stages of germination differed antigenically from the α-amylases found in developing barley seeds. The relation between the levels of gibberellic acid and the production and activity of α-amylases in barley has been studied.[205]

Malt amylase (a commercial, impure mixture of α- and β-amylases from barley malt) has been used for the hydrolysis of starch in a membrane reactor.[206] The system was based on the respective enzymes having molecular weights of 6.0×10^4 and 19.7×10^4, since both were rejected by the membrane, which had a cut-off point at 1.8×10^4. The membrane reactor gave a better performance than a solid-wall reactor only over extended periods, whereas a membrane with a sharper cut-off was needed for short times.

Three to eight isoenzymes of amylase have been detected in the chloroplasts of leaves of four varieties of cotton plants (*Gossypium* genus).[207]

α-Amylase activity was observed in the roots of a plant (*Verbascum thapsus*) requiring low temperatures for growth.[208] The α-amylase exhibited maximum activity at 15 °C, and a further increase in the activity was apparent when, after being kept at 4 °C, the plant commenced growing at higher temperatures.

The α-amylase components of amylolytic systems present in *Aspergillus cinnamomeus* and a mutant thereof have been isolated by ion-exchange chromatography.[209] The molecular sizes were assessed by gel filtration, and the relative activities in the two strains were determined.

The effects of pH, heat, and H_4edta on Taka-amylase (one of the α-amylases of *Aspergillus oryzae*) have been determined; the enzyme was inhibited by H_4edta.[210]

Over 200 strains of *Bacillus* species have been scanned for α-amylase activity.[211] Enzyme preparations and fractions obtained from gel electrofocusing of different samples of *B. subtilis* exhibited identical action patterns in the hydrolyses of amylose and phenyl α-maltoside.[212] The kinetics of α-amylases from two strains of *B. subtilis* showed little dependence upon the degree of polymerization of the substrate in the range 3—320.[213] The number of effective subsites in the active sites of the enzymes was determined and their affinities were evaluated from the dependence of k_0/K_m upon the degree of polymerization. The subsite affinities of both enzymes were found to be similar to each other and to those of another bacterial α-amylase. The modes of cleavage of malto-oligosaccharides were in agreement with those evaluated from the subsite affinities.

In the absence of Ca^{2+} or Zn^{2+} ions, the molecular weight (4.8×10^4, by gel filtration and ultracentrifugation) of the α-amylase from *B. subtilis* was independent of pH and concentration.[214] In solutions of guanidinium hydrochloride, the molecular weight was reduced to 2.35×10^4, indicating the presence of two

[205] G. H. Palmer, *J. Inst. Brewing*, 1973, **79**, 513.
[206] E. Tachauer, J. T. Cobb, and Y. T. Shah, *Biotechnol. Bioeng.*, 1974, **16**, 545.
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[213] S. Iwasa, H. Aoshima, K. Hiromi, and H. Hatano, *J. Biochem. (Japan)*, 1974, **75**, 969.
[214] E. D. Mitchell, P. Riquetti, R. H. Loring, and K. L. Carraway, *Biochim. Biophys. Acta*, 1973, **295**, 314.

subunits of equal size. C.d. spectroscopy showed that the molecule adopts the random-coil conformation in this solution. The molecular weight (1.54×10^5) determined by electrophoresis in sodium dodecyl sulphate is anomalous; studies of the binding by equilibrium dialysis showed that native α-amylase bound less detergent than the denatured enzyme. A cross-reacting material serologically related to α-amylase has been obtained from cultures of *B. subtilis* by precipitation, ion-exchange chromatography, gel filtration, and disc electrophoresis.[215] This homogeneous material was devoid of enzymic activity, but possessed the same molecular weight, thermal stability, electrophoretic mobility, and immunological reactivity as the α-amylase from the organism; it contains neutral carbohydrate (8%) and 2-amino-2-deoxy-D-glucose (6%) in amounts differing from those of the enzyme (7 and 2%, respectively). These and other results indicated that the mutant giving rise to this material has a small inframe deletion or inversion.

The amylase from *Rhizopus javanicus* has been shown to be a glycoprotein containing D-mannose (27) and 2-acetamido-2-deoxy-D-glucose (4 moles per mole).[216] Three glycopeptides isolated from pronasic digests of the heat-denatured amylase were composed of asparagine, glycine, D-mannose, and 2-acetamido-2-deoxy-D-glucose (1 : 1 : 6 : 2), asparagine, threonine, D-mannose, and 2-acetamido-2-deoxy-D-glucose (1 : 1 : 9 : 2), and threonine, serine, proline, alanine, and D-mannose (6 : 2 : 2 : 2 : 12).

Active immobilized forms of α-amylase have been prepared by reaction of the enzyme with cyclic imidocarbonate derivatives of agarose and Sephadex gels,[210] and by irradiation of the enzyme in the presence of acrylamide.[80]

β-Amylases

The ability of purified, pirkka-barley β-amylase to polymerize has been studied by a new electrophoretic technique, using an acrylamide-gradient gel, which confirmed that the enzyme polymerized spontaneously in the absence of a reducing agent.[217] Estimations of the molecular weights indicated that the polymers comprise several monomeric forms of the enzyme, each of a slightly different molecular size.

Electrophoresis of amylase from the starchy endosperm of barley (*Hordeum vulgare*) seeds yielded nine β-amylases; four of them are isoenzymes (molecular weights 4.3×10^4), whereas the other five are molecular aggregates of these monomers.[203] A dimer, a tetramer, and an octamer (molecular weights 8.6×10^4, 1.8×10^5, and 4.0×10^5, respectively) were identified. Five additional forms of β-amylase, which were not recognized by electrophoresis, possess molecular weights of $4—30 \times 10^3$. An *endo*-acetamidodeoxy-D-glucosidase obtained from crude barley β-amylase was found to retain β-amylase activity after repeated isoelectric focusing.[185]

The continuous hydrolysis of starch by sweet potato amylase in a tubular membrane unit has been studied at different concentrations of substrate and enzyme for a given pressure and inflow.[218] The performance of the membrane

[215] K. Yamane, K. Yamaguchi, and B. Maruo, *Biochim. Biophys. Acta*, 1973, **295**, 323.
[216] K. Watanabe and T. Fukimbara, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2755.
[217] M.-L. Niku-Paavola, A. Skakoun, M. Nummi, and J. Daussant, *Biochim. Biophys. Acta*, 1973, **322**, 187.
[218] G. P. Closset, J. T. Cobb, and Y. T. Shah, *Biotechnol. Bioeng.*, 1974, **16**, 345.

reactor was better than that of a solid-wall reactor. Ageing of the starch solution and the formation of a gel at the surface of the membrane reduced the efficiency of the system.

The existence of five variant types of zymograms only for the β-amylase from *Triticum aestivum* in numerous varieties of the wheat has been demonstrated, and the zymograms were therefore proposed as genetic markers.[219]

β-Amylase from wheat bran has been purified to a single protein component (as determined by ultracentrifugation and polyacrylamide gel electrophoresis), which was free from α-amylase activity.[220] The kinetics of hydrolysis of linear substrates were studied, and it was concluded that the enzyme has five subsites. Values of k_0/K_m and K_m were used to evaluate the affinity of each subsite, and the results indicated that maltotetraose is the smallest effective substrate for the enzyme, whereas maltose and phenyl α-maltoside are not hydrolysed to any appreciable extent.

β-Amylase activity has been observed in the roots of a plant (*Verbascum thapsus*) requiring relatively low temperatures for growth.[208] The enzyme displayed maximum activity at 15 °C, but did not appear to be directly concerned with the growth of shoots following vernalization.

Over 200 strains of *Bacillus* species have been scanned for β-amylase activity.[211] The effects of the concentrations of starch and polypepton on the formation of β-amylase by *Bacillus* sp. *BQ10* were investigated; this enzyme exhibited maximum activity at 58 °C and pH 6.5, whereas the β-amylase from a *Streptomyces* species exhibited maximum activity at 40 °C and pH 6.5.

Active immobilized derivatives of β-amylase have been prepared by irradiation of the enzyme in the presence of acrylamide [80] and by covalent attachment to a cross-linked copolymer of acrylamide and acrylic acid.[221] The last procedure was also used to immobilize β-amylase and pullulanase within the same matrix.[222]

Arabinanases

The induction of cell-wall-degrading enzymes has been studied in vascular wilt fungi (*Fusarium oxysporum* and *Verticillium albo-atrum*) grown with restricted supplies of a number of cell-wall sugars. L-Arabinose and, to a lesser extent, D-galactose were able to induce arabinase activity.[125]

An extracellular hemicellulase from *Bacillus pumilus* cultured in the presence of soybean has been purified by ion-exchange chromatography and gel filtration.[223] The enzyme (pH optimum 5.5, temperature optimum 50 °C) hydrolysed hemicellulose to give two products, which both gave D-galactose and L-arabinose on hydrolysis with acid.

Cellulases

An assay has been reported for cellulase that measures the amount of reducing sugar (expressed as D-glucose) liberated from Whatman No. 1 paper.[224] The

[219] P. Joudrier, *Compt. rend.*, 1974, **278**, *D*, 1777.
[220] M. Kato, K. Hiromi, and Y. Morita, *J. Biochem.* (*Japan*), 1974, **75**, 563.
[221] K. Mårtensson, *Biotechnol. Bioeng.*, 1974, **16**, 567.
[222] K. Mårtensson, *Biotechnol. Bioeng.*, 1974, **16**, 579.
[223] K. Kiuchi, T. Ohta, N. Kato, and H. Ebine, *J. Food Sci. Technol.*, 1973, **20**, 239.
[224] H. L. Griffin, *Analyt. Biochem.*, 1973, **56**, 621.

effects of time and the concentration of the substrate on cellulase activity were studied, and it was assumed that the reducing sugar arises from the action of cellulase on the more susceptible portions of the substrate.

A review of active-site-directed inhibitors and the mechanisms of action of carbohydrases has discussed the characteristics of cellulase.[14]

Purified cellulase from workers of the termite *Trinervitermes trinervoides* readily attacked insoluble cellulose, and the effect of the physical state of the substrate on its rate of hydrolysis was investigated.[225] The cellulase exhibited a random pattern of hydrolysis, furnishing cellotriose as the principal product.

A cellulase complex obtained from 'post breaker' tomato (*Lycopersicon esculentum*) has been shown to contain β-D-glucosidase, *exo*-β-1,4-glucanase, and two *endo*-cellulases having pH optima 4.2, 4.2, 5.2, and 7.5, respectively.[148] Insoluble cellulose was degraded to short-chain cellodextrins and D-glucose by the joint action of these enzymes.

Cotyledons of germinating kidney beans (*Phaseolus vulgaris*) contain two forms of cellulase that were separated by precipitation with ammonium sulphate and isoelectric focusing.[226] Both forms are of similar molecular weight, but their isoelectric points, pH and temperature optima, pH and thermal stabilities, and sensitivities to thiol inhibitors and metal ions differ. One form of cellulase (pI 4.5) is present in both abscissing and non-abscissing tissues of *P. vulgaris*, and required grinding prior to extraction, whereas another form (pI 9.5), which could be easily removed with buffer, is present in tissues in which abscission had been induced.[227] The intracellular location of the two forms of cellulase was discussed.

Cellulase has been extracted from solid cultures of a number of strains of *Aspergillus oryzae* and *A. sojae* grown on wheat bran.[228] Species-specific patterns were observed on polyacrylamide gel electrophoresis, but no variation was noted between strains of the same species. The use of electrophoretic zymograms was suggested as an aid to taxonomy.

The induction of cell-wall-degrading enzymes has been studied in vascular wilt fungi (*Fusarium oxysporum* and *Verticillium albo-atrum*) grown with restricted supplies of a number of cell-wall sugars; cellobiose was particularly effective in inducing cellulase activity.[125]

A commercial enzyme preparation obtained from a *Flavobacterium* species was found to contain a number of enzymes, including a cellulase, specific for glucans.[229]

Growth of *Geotrichum candidum* on by-products of sugar beet, wheat bran, *etc.* produced enzymes that hydrolysed cellulose to D-glucose.[230] Ion-exchange chromatography separated the enzyme into C_1 (molecular weight 6.46×10^4) and C_x (molecular weight 5.17×10^4) components. Whereas a combination of the C_1 and C_x components was effective in degrading cotton fibres, cellulases

[225] R. C. Potts and P. H. Hewitt, *Comp. Biochem. Physiol.*, 1974, **47B**, 327.
[226] F. T. Lew and N. Lowell, *Phytochemistry*, 1974, **13**, 1359.
[227] P. D. Reid, H. G. Strong, F. Lew, and L. N. Lewis, *Plant Physiol.*, 1974, **53**, 732.
[228] S. Nasuno, *Canad. J. Microbiol.*, 1974, **20**, 413.
[229] D. J. Manners and G. Wilson, *Biochem. J.*, 1973, **135**, 11.
[230] N. A. Rodionova, N. A. Tiunova, R. V. Feniksova, T. I. Kudriashova, and L. I. Martinovich *Doklady Akad. Nauk S.S.S.R.*, 1974, **214**, 1206.

from fungi with high activities against carboxymethylcellulose did not degrade cotton fibres, even in the presence of the C_1 component.

Strains of *Mucor miehei* and *M. pusillus* have been found to synthesize cellulase when cultured on a medium containing a suspension of wheat bran.[153] The inducible enzyme complex hydrolysed carboxymethylcellulose, and acid-swollen and unmodified celluloses. The *exo-* and *endo*-activities of the cellulase on various forms of cellulose in the presence and in the absence of the endogenous β-D-glucosidase were compared.

Various culture media have been examined as potential substrates for the commercial production of cellulase from *Myrothecium verrucaria*, *Stachybotrys atra*, and *Trichoderma viride*.[231] Of the cellulosic inducers of the enzyme examined, filter paper was the most effective, and sugar cane, bagasse, and carboxymethylcellulose less so.

The effect of the cellulase produced by *Penicillium funiculosum* on various crystalline celluloses has been investigated.[232] Differences in the responses were attributed to variations in particle size, in surface area, in crystallinity, and in the dimensions of the crystallites. *X*-Ray diffraction revealed that the crystallinity of the substrate increased by 7—10% on enzymic hydrolysis, with no appreciable change in the dimensions of the crystallites, indicating that the enzyme's action was confined primarily to the paracrystalline regions of the surface.

The induction by cellulose of additional cellulase activity in the wood-rotting fungus *Polyporus adustus* was followed by the appearance of mannanase and xylanase activities; it was suggested that all the enzymes are under the control of a single, common, regulatory gene.[233]

Culture media of *Pseudomonas fluorescens* have been shown to contain a number of cellulase components of different molecular weights, and variations of the relative concentrations of these components with time were compared.[234] The relationship of the components to the formation of the enzyme complex within the cell was also considered.

Cellulase obtained by precipitation with ammonium sulphate from culture filtrates of various strains of *Pyricularia oryzae* grown on rice-plant powder has been fractionated by ion-exchange chromatography into adsorbed and non-adsorbed components.[235] The components were further fractionated by gel filtration; the activities of several of the peaks varied according to the strain used, whereas others were common to all strains.

The cellulolytic C_1 component from *Trichoderma koningii* has been freed from activities associated with the cellulase complex, and was shown to release cellobiose from simple and complex forms of native cellulose.[154] The extent of enzymic action was determined by the nature of the substrate, but the C_x component of the cellulase system was not required for activity. The enzyme synergized extensively with cellobiase to extend the hydrolysis of celluloses up to 70%. The enzyme was relatively unstable, and was inactivated by its product

[231] H. C. de Menezes, T. J. B. de Menezes, and H. V. Boas, *Biotechnol. Bioeng.*, 1973, **15**, 1123.
[232] S. M. Betrabet, V. G. Khandeparkar, and N. B. Patil, *Cellulose Chem. Technol.*, 1974, **8**, 339.
[233] K. E. Eriksson and E. W. Goodell, *Canad. J. Microbiol.*, 1974, **20**, 371.
[234] T. Yoshikawa, H. Suzuki, and K. Nisizawa, *J. Biochem.* (*Japan*), 1974, **75**, 531.
[235] T. Sudo, H. Nagayama, and K. Tamari, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2535.

(from which it was released by cellobiase) and by Cu^{2+}, Zn^{2+}, Fe^{3+} ions, and phenylmercury, but not by D-glucose. The relation of the enzyme to the entire cellulase complex was discussed.

Extracellular filtrates of *Trichoderma viride* showed considerable cellulolytic activity against native celluloses, cellulose derivatives, and raw and waste materials.[236] Yellow paper and rice straw were preferentially attacked by the enzyme.

Chitinases

Enzyme preparations from porcine and murine organs released 2-[^{14}C]-acetamido-2-deoxy-β-D-glucopyranosyl-L-asparagine from a labelled version of glycopeptide (1), without the formation of intermediate products.[237] The purified porcine-liver enzyme was free from α-mannosidase activity, and its action pattern appeared to be unique, since it did not hydrolyse glycopeptides other than those derived from ovalbumin.

D-Man*p*-β-D-Man*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn

(1)

Two chitinases have been detected in haemolymph and cell cultures of the cockroach (*Periplaneta americana*); the purified enzymes showed identical chromatographic and electrophoretic behaviours, and possessed no lytic activity towards cell suspensions of *Micrococcus lysodeikticus*.[238] Haemolymph collected from females carrying ootheca was devoid of chitinase activity.

Isotherms for the adsorption of a chitinase from a *Streptomyces* species onto chitin have been determined at various temperatures.[239] The initial rate of hydrolysis of chitin was proportional to the total concentration of enzyme.

The chitinase activities of a number of organisms have been monitored and compared with the chitosanase activities thereof.[240]

Chitosanases

Distinct chitosanase activities have been detected in a number of organisms, including species of *Actinomyces*, *Arthrobacter*, *Aspergillus*, *Bacillus*, *Beuveria*, *Cokeromyces*, *Gliocadium*, *Metarrhizium*, *Penicillium*, *Rhizopus*, *Rhodotorula*, *Serratia*, *Stachybotrys*, *Trichurus*, *Trichoderma*, and *Zygorrhynchus*.[240] The organisms were grown on dead cells of *Rhizopus rhizopodiformis*, and the lysis of living cells of this organism was used to determine the chitosanase activities. In many instances the chitosanase activity exceeded the chitinase activity.

Chondroitin Sulphate Hydrolases

[^{14}C]Chondroitin sulphate was degraded to a material of molecular weight 2000 by an enzyme from embryonic chick cartilage.[241] The action of the enzyme

[236] J. K. Gupta, Y. P. Gupta, and N. B. Das, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2657.
[237] M. Nishigaki, T. Muramatsu, and A. Kobata, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 638.
[238] I. Bernier, J.-C. Landureau, P. Grellet, and P. Jollès, *Comp. Biochem. Physiol.*, 1974, **47B**, 41.
[239] J. Skujins, A. Pukite, and A. D. McLaren, *Mol. Cell. Biochem.*, 1973, **2**, 221.
[240] R. L. Monaghan, D. E. Eveleigh, R. P. Tewari, and E. T. Reese, *Nature New Biol.*, 1973, **245**, 78.
[241] R. Amadò, B. Ingmar, U. Lindahl, and Å. Wateson, *F.E.B.S. Letters*, 1974, **39**, 49.

was impeded in the absence of sodium chloride, and, although the enzyme exhibited maximum activity at pH 2.3, it was also active at pH 1.5. No *exo*-glycosidase activity was evident at these pH values, although it was present, and the *endo*-activity was absent, at pH 4.5. It was concluded that the substrate is degraded *in vivo* by an *endo*-enzyme, followed by the actions of *exo*-enzymes and a sulphatase.

Dermatan Sulphate Lyases

Flavobacterium heparinum grown on chondroitin 4- or 6-sulphates or dermatan sulphate produced a dermatan sulphate lyase which could be separated from the constitutional chondroitin lyase AC.[242] The enzyme degraded dermatan sulphate to 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranuronosyl)-D-glucose and higher oligosaccharides, which were resistant to dermatan sulphate lyase but susceptible to chondroitin lyase AC.

Dextranases

A novel kind of dextranase has been isolated from the supernatant of cultures of an *Achromobacter* species.[243] The enzyme hydrolysed dextran to isomaltose by an *exo*-action without the liberation of other oligosaccharides. A high-molecular-weight limit dextrin resisted the action of the enzyme, but was susceptible to a dextranase from *Penicillium luteum*. The new dextranase hydrolysed panose to isomaltose and D-glucose, and isomaltotri-itol to isomaltose and D-glucitol.

Of 44 fungi isolated from soil samples, none produced dextranase in appreciable amounts, but one (*Aspergillus luchuensis*) of seven isolated from air produced dextranase in high yield.[244] During the production of dextranase by fermentation, determination of the *exo*-activity (by measuring the reducing properties) and *endo*-activity (by measuring the reduction in viscosity) towards dextran gave comparable results.

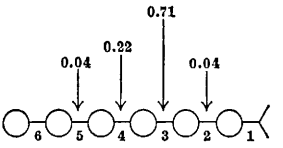
Numerous strains of dextranase-producing organisms have been examined in some detail for the pH–activity relationship and the stability of the enzyme.[245] Cultures of a *Brevibacterium* species produced an 'alkaline' dextranase, and optimal conditions for production of the enzyme were established. This thermostable enzyme had a pH optimum of 8.0 at 37 °C, an optimum temperature of 53 °C at pH 7.5, and was stable up to pH 10.5.

A dextranase from *Brevibacterium fuscum* has been purified by gel filtration and ion-exchange chromatography to give a single band on disc electrophoresis.[246] The enzyme (pH optimum 7.0—7.5) was stable in the pH range 5.0—11.0 and was activated by L-cysteine and H_4edta, but was inactivated by iodine, mercury(II) chloride, *N*-bromosuccinimide, and copper(II) sulphate. The dextranase was of the *exo*-type and liberated isomaltotriose only from dextran.

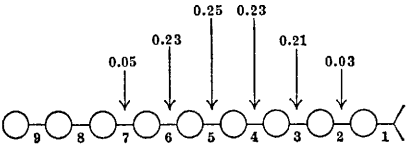
The products of the action of a purified, extracellular *endo*-dextranase from a *Pseudomonas* species on isomalto-oligosaccharides have been examined; reduced

[242] Y. M. Michelacci and C. P. Dietrich, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 973.
[243] T. Sawai, K. Toriyama, and K. Yano, *J. Biochem.* (*Japan*), 1974, **75**, 105.
[244] V. K. Joshi and D. V. Tamhane, *Current Sci.*, 1973, **42**, 720.
[245] T. Yamaguchi and S. Gocho, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2527.
[246] M. Sugiura, A. Ito, and T. Yamaguchi, *Biochim. Biophys. Acta*, 1974, **350**, 61.

and tritiated isomalto-oligosaccharides were also used as substrates.[247] The patterns of cleavage of reduced dextran hepta- and deca-saccharides are shown in Schemes 2 and 3, respectively. The explanation for the formation of relatively



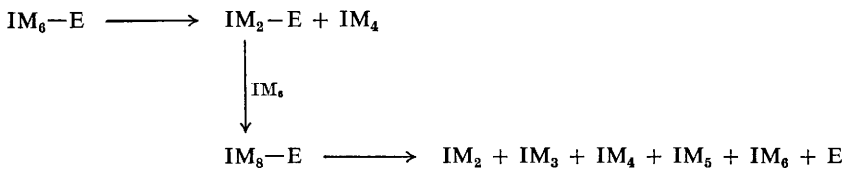
Scheme 2



Scheme 3

○, −, and ⟨ represent a D-glucose residue, an α-(1 → 6) linkage, and the terminal alditol group, respectively.

large amounts of the pentasaccharide from the hexasaccharide, without the liberation of D-glucose, is summarized in Scheme 4. On the basis of these and other results, a model was proposed for the active site of the enzyme.



E = Enzyme.
IM_n = isomalto-oligosaccharide

Scheme 4

Galactanases

An enzyme capable of hydrolysing galactomannans has been obtained from germinating seeds of *Cyamopsis tetragonoloba*.[248] The enzyme (pH optimum 4.4 at 35 °C, K_m 20 mg ml^{-1}) was inhibited appreciably by D-galactose, whereas D-mannose had a negligible effect. Such plant-growth regulators as gibberellic acid and cyctocel inhibited the enzyme's activity.

An extracellular hemicellulase produced by *Bacillus pumilus* cultured in the presence of soybean has been purified by ion-exchange chromatography and gel filtration.[223] The enzyme (pH optimum 5.5, temperature optimum 50 °C) hydrolysed hemicellulose to give two products, both of which yielded D-galactose and L-arabinose on hydrolysis with acid.

An enzyme, possibly an *endo*-β-D-galactosidase, has been obtained from a *Coccobacillus* species and was purified by ion-exchange chromatography and gel filtration.[116] The enzyme (pH optimum 5.5, temperature optimum 32 °C) was characterized as a basic protein which degraded keratan sulphate to a series of sulphated oligosaccharides and glycopeptides. Although the enzyme was inhibited by heavy-metal ions, it was unaffected by H_4edta or dithiothreitol. The enzyme's activity was measured by the turbidity produced on reaction of

[247] G. N. Richards and M. Streamer, *Carbohydrate Res.*, 1974, **32**, 251.
[248] K. Sehgal, H. S. Nainawatee, and B. M. Lal, *Biochem. Physiol. Pflanzen*, 1973, **164**, 423.

the residual keratan sulphate with hyamine. The enzyme also hydrolysed other glycoproteinaceous material.

endo-β-1,3-Glucanases

Although gibberellic acid increased the level of the *endo*-β-1,3-glucanase in barley, this enzyme, unlike the *endo*-β-glucanase present, developed to significant levels in the absence of gibberellic acid.[205] The actions of malted-barley *endo*-β-1,3-glucanases on barley and malt glucans differ.[249] Malt glucan was rapidly hydrolysed with a decrease in the specific viscosity of the solution, but without a measurable increase in the reducing power, whereas barley glucan underwent only limited hydrolysis under comparable conditions. An enzyme from a *Flavobacterium* species has been prepared by ion-exchange chromatography (see ref. 248), but, contrary to an earlier report (Vol. 7, p. 429, ref. 261), the unadsorbed *endo*-β-1,3-glucanase liberated D-glucose, as well as oligosaccharides, from laminarin.

Continuous electrophoresis of a commercial enzyme preparation obtained from a *Flavobacterium* species yielded a protein that hydrolysed laminarin, carboxymethylpachyman, barley β-glucan, lichenin, and cellodextrin in random fashion.[229] The activity of the unstable enzyme preparation was ascribed to the presence of an *endo*-β-1,3-glucanase and three other glucan-hydrolysing enzymes.

endo-β-1,6-Glucanases

The hydrolysis of linear poly- and oligo-saccharides containing β-(1 → 3)- and β-(1 → 6)-D-glucosidic linkages by a purified *endo*-β-1,6-glucanase from *Gibberella fujikuroi* has been studied.[250] 3^2-β-Gentiobiosylgentiobiose was hydrolysed to gentiobiose, while 3^2-β-gentiotriosylgentiobiose gave gentiobiose, gentiotriose, and 6-β-laminaribiosyl-D-glucose. The initial concentrations of lutean, gentiotetraose, and 3^2-β-gentiobiosylgentiobiose giving apparent maximal rates of hydrolysis by the enzyme were 0.0015, 0.28, and 3.4 mmol l^{-1}, respectively. The substrate specificity of the *endo*-β-1,6-glucanase was discussed.

Glucanases (Miscellaneous)

Barley *endo*-β-glucanase has been found to require a higher concentration of gibberellic acid to maximize its production than does the α-amylase, and the *endo*-β-glucanase does not develop to a significant level in the absence of gibberellic acid.[205] In aleurone layers treated with gibberellic acid, β-glucanases degraded the cell wall mainly to D-glucose. D-Xylose and cellobiose appeared when the aleurone layers had undergone extensive enzymic hydrolysis, and laminaribiose and L-arabinose were found whether gibberellic acid was present or not. In addition to degrading the endosperm layers, β-glucanases may be important in releasing enzymes from the aleurone layers into the endosperm during malting.

The independent biosynthesis of glucanases in the complex of lytic enzymes produced by thermotolerant *Actinomyces griseinus* has been investigated, and the

[249] G. N. Bathgate, G. H. Palmer, and G. Wilson, *J. Inst. Brewing*, 1974, **80**, 278.
[250] Y. Shibata, *J. Biochem.* (*Japan*), 1974, **75**, 85.

effect of the conditions of growth on the production of the enzymes was established.[251]

An assay for the maltohexaose-producing amylase of *Aerobacter aerogenes* has been based on the use of reduced, short-chain amylose (DP 20) as a substrate and an automated ferricyanide reaction.[12] The assay was shown to be selective for the amylase in the presence of pullulanase and other debranching enzymes.

The action pattern of a lytic β-1,3-glucanase isolated from an *Arthrobacter* species has been studied; the enzyme liberated laminaripentaose from various β-glucans.[252] The enzyme did not degrade short, linear laminaridextrins, but was active towards a linear β-1,3-oligoglucan synthesized enzymically. Intact D-glucosyl residues at the chain-ends of a substrate did not appear to be a prerequisite for the action of the enzyme.

An enzyme that depolymerized succinoglucan has been purified from culture fluids of a *Flavobacterium* species.[253] The enzyme (molecular weight 1.8×10^5, pH optimum 5.8) was stable up to 35 °C and in the pH range 4.5—10.0 (K_m 1.7 and 1.2 mg ml^{-1} for succinoglucan and desuccinylated succinoglucan, respectively). Besides succinoglucan, which appeared to be hydrolysed to its monomeric units, only yeast glucan and pachyman were hydrolysed, and then only slightly. The enzyme was formed only when the succinoglucan and the desuccinylated derivative thereof were present, and such compounds as D-glucose and succinic acid repressed the formation of the enzyme.

Two of the components of a commercial enzyme preparation from a *Flavobacterium* species, which hydrolysed laminarin, carboxymethylpachyman, barley β-glucan, lichenin, and cellodextrin in random fashion, were identified as β-glucanases of high and low molecular weight.[229]

The production of β-1,3-glucanase by a thermophilic species of *Streptomyces* grown in a chemically defined medium has been studied.[254] Synthesis of the extracellular enzyme is semiconstitutive and subject to catabolite repression by metabolizable carbon substrates. The enzyme was induced by gentiobiose.

Glucoamylases

A form of glucoamylase obtained from *Aspergillus awamori* was shown by sedimentation equilibrium studies to possess a molecular weight of 6.2×10^4.[255] Compositional studies were carried out, and the *N*-terminus was identified as L-valine. The enzyme hydrolysed starch, amylose, amylopectin, and glycogen at twice the rate of maltose and isomaltose.

Glucoamylases I and II have been purified by ion-exchange chromatography from amylolytic systems of *Aspergillus cinnamomeus* and a mutant thereof obtained by u.v.-irradiation.[209] The glucoamylase activities were stronger in the mutant strain than in the parent.

Rate parameters for the hydrolysis of phenyl 6-*O*-acetyl-α-maltopyranoside by glucoamylase from *Rhizopus delemar* have been determined; whereas values for

[251] S. A. Konovalov, S. P. Vorotilo, and L. A. Ziukova, *Microbiologia*, 1974, **43**, 261.
[252] K. Doi, A. Doi, T. Ozaki, and T. Fukui, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1629.
[253] A. Amemura, K. Moori, and T. Harada, *Biochim. Biophys. Acta*, 1974, **334**, 398.
[254] G. Lilley and A. T. Bull, *J. Gen. Microbiol.*, 1974, **83**, 123.
[255] S. V. Durmishidze, G. I. Kvesitadze, and G. N. Kokonashvili, *Doklady Akad. Nauk S.S.S.R.*, 1974, **217**, 470.

the activity were similar to those for maltose and phenyl α-maltopyranoside, the K_m value was appreciably higher.[256] The acetylated maltoside was used as a substrate for inhibition studies with monosaccharides, glycosides, and disaccharides, which proved to be competitive inhibitors. The molecular binding affinity (*i.e.* the unitary free-energy decrement due to formation of the enzyme–inhibitor complex) was analysed quantitatively with respect to the structures involved.

Active immobilized forms of glucoamylase have been prepared by covalent attachment of the enzyme to an arylamine derivative of glass [257] and by irradiation in the presence of acrylamide.[80]

exo-β-D-1,4-Glucosidase

An *exo*-β-D-1,4-glucosidase (pH optimum 4.2), which completely degraded cellulose, has been identified among the polysaccharide hydrolases of 'post breaker' tomato (*Lycopersicon esculentum*).[148] The enzyme complex is analogous to that found in cellulolytic bacteria and fungi.

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.[258] The heparin lyase acted on heparin and heparan sulphate to give a trisulphated disaccharide, whereas one of the heparan sulphate lyases gave two *N*-acetylated disaccharides, one of which was also *O*-sulphated. The other heparan sulphate lyase degraded heparan sulphate to an *N*,*O*-disulphated disaccharide. The modes of action of these enzymes were discussed.

Hyaluronidases

Bovine testicular hyaluronidase (molecular weight 6.0×10^4), which gave a single band on gel electrophoresis in the presence of sodium dodecyl sulphate, did not dissociate into subunits under more drastic conditions.[259] Samples treated with urea or maleic anhydride gave only faint bands (molecular weight 3.7×10^4), but there was no evidence that the enzyme is composed of subunits. Studies of the binding of a tetrasaccharide, obtained by the action of the enzyme on hyaluronic acid, suggested that the enzyme possesses only one binding site for the tetrasaccharide.

Acrosomal hyaluronidase (pH optimum 3.75) has been partially purified from extracts of bovine spermatozoa by ion-exchange chromatography.[260] The stability of the enzyme was investigated; it was inhibited by Fe^{2+} and Fe^{3+} ions and heparin, but not by Mn^{2+}, Mg^{2+} ,and Ca^{2+} ions or H_4edta. A partially purified hyaluronidase (molecular weight 1.1×10^5) obtained from rabbit

[256] K. Hiromi, M. Kawai, N. Suetsugu, Y. Nitta, T. Hosotani, A. Nagao, F. Nakajima, and S. Ono, *J. Biochem.* (*Japan*), 1973, **74**, 935.
[257] H. H. Weetall, N. B. Havewala, H. M. Garfinkel, W. M. Buehl, and G. Baum, *Biotechnol. Bioeng.*, 1974, **16**, 169.
[258] M. E. Silva and C. P. Dietrich, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 965.
[259] J. H. Garvin and D. M. Chipman, *F.E.B.S. Letters*, 1974, **39**, 157.
[260] L. J. D. Zanefield, K. L. Polakoski, and G. F. B. Schumacher, *J. Biol. Chem.*, 1973, **248**, 564.

spermatozoa possessed similar properties. Immunological studies suggested that acrosomal hyaluronidase is identical with testicular hyaluronidase, but that it differs from lysosomal hyaluronidase.

The release of hyaluronidase from capacitating hamster spermatozoa has been measured by a new sensitive assay that is based on the higher rate of hydrolysis of a hyaluronic acid–albumin complex than of hyaluronic acid by the enzyme.[261]

Nearly all the hyaluronidase activity of rat kidney was found to be associated with the cortex, from which a hyaluronidase-rich extract was obtained.[262] The enzyme (pH optimum 3.7) was inhibited by copper(II) acetate. Hyaluronidases in the lysosomes of rat liver and hepatoma have also been investigated.[263]

The hyaluronidase of rat-sperm acrosomes was released by hyamine and Triton, and was separated from acrosin by gel filtration.[264] The enzyme (molecular weight 6.2×10^4, pH optimum 4.3), further purified by ion-exchange chromatography, was inhibited by heparin and dermatan sulphate; it was also examined by gel electrophoresis in the presence of sodium dodecyl sulphate.

Keratan Sulphate Hydrolases

An enzyme obtained from a *Coccobacillus* species by ion-exchange chromatography and gel filtration was found to hydrolyse keratan sulphate to oligosaccharides and glycopeptides.[116] The enzyme activity was measured by reaction of the remaining keratan sulphate with hyamine and determination of the turbidity produced. The activity of this basic enzyme (pH optimum 5.5, temperature optimum 32 °C) was inhibited by heavy-metal ions, but by neither H_4edta nor dithiothreitol.

Laminarinases

Laminarinase activity has been found in homogenates of workers of the termite *Trinervitermes trinervoides*.[225]

A purified enzyme from *Gibberella fujikuroi* hydrolysed a linear laminarin, containing both β-1,3- and β-1,6-linkages, to 3^2-β-gentio-oligosylgentiobioses, while 3^2-β-gentiobiosylgentiobiose was hydrolysed to gentiobiose.[250] The initial concentrations of the poly- and oligo-saccharides giving apparent maximum velocities were compared.

In a study of the sequential actions of cell-wall hydrolases on the germination and outgrowth of *Microsporum gypseum* macroconidia, it was found that laminarinase activity plays a part in initiating germination.[65] This and other hydrolases are compartmentalized in lysosomal vesicles, which appear to be delivered to the germinating spores in a co-ordinated manner.

An extracellular laminarinase has been isolated from *Rhizopus arrhizus* and, although the enzyme (molecular weight 10^4) was unstable in dilute solution, it could be stabilized with human serum albumin.[265] The kinetics of the hydrolyses

[261] B. J. Rogers and B. E. Morton, *J. Reprod. Fert.*, 1973, **35**, 477.
[262] J. Komender, H. Malczewska, and A. Golaszewska, *Bull. Acad. polon. Sci., Sér. biol. Sci.*, 1973, **21**, 637.
[263] B. F. Szafarz and D. Szafarz, in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972, p. 737.
[264] C. H. Yang and P. N. Srivastava, *J. Reprod. Fert.*, 1974, **37**, 17.
[265] J. J. Marshall, *Carbohydrate Res.*, 1974, **34**, 289.

of laminarin and lichenin were studied, and the results of detailed stability tests with serum albumin and ammonium molybdate indicated the presence of two enzymes (pH optima 3.5 and 4.6).

Lysozymes

A light-hearted poem on lysozymes and lysosomes compares the attributes of the two entities, and is directed towards the confusion that frequently arises between the two terms.[266] A definitive and comprehensive work on lysozyme has reviewed the entire field, including fundamental chemical, biochemical, and biophysical investigations, as well as physiological and clinical aspects.[267] A review of active-site-directed inhibitors and the mechanisms of action of carbohydrate hydrolases has dealt with lysozyme specifically.

Lysozyme has been isolated from human milk and saliva by affinity chromatography on an immobilized form of rabbit anti-(hen egg-white lysozyme).[268] However, neither whey nor saliva gave a precipitate with the antibody, suggesting immunological similarities between the two lysozymes and the avian form. The preparation of lysozyme from human milk and echidna, and the occurrence of lysozyme activities in the milk of cows, pigs, horses, red kangaroos, and platypuses have been reported.[269] The amino-acid compositions of the lysozymes and α-lactalbumins of a number of species were compared, and multiple forms of the lysozymes were detected. One of them, an echidna lysozyme, exhibited lactose-synthetase modifier activity in the presence of bovine A-protein. The significance of lysozymes and α-lactalbumins was discussed.

The purification, composition, and fluorescence and c.d. spectra of rat lysozyme have been reported.[270] Fluorescence-remission and other spectral data, the effect of pH, and the inhibition by tetra-*N*-acetylchitotetraose paralleled those of a human lysozyme. Further discussion of the spectral properties was based on amino-acid sequences known to occur in lysozymes.

The rate of hydrolysis of hexa-*N*-acetylchitohexaose by hen egg-white lysozyme has been determined by measuring the increase in reducing sugar and by charcoal-column analysis of the products of transglycosylation.[271] At all pH values studied, only one bond in five was cleaved, and the kinetics were first-order, indicating equally strong binding of the substrate with the tetrasaccharide produced. Further studies of the free and non-productive enzyme–hexamer complexes gave pK values of 6.1 and 6.7 for glutamic acid-35 and 3.4—3.7 and 3.8 for aspartic acid-52, respectively.

Pentane inhibited the lysis of *Micrococcus lysodeikticus* by egg-white lysozyme, but only when added to a solution of the enzyme prior to mixing with the cells.[272] The hydrolysis of glycolchitin by the enzyme was not inhibited by pentane, suggesting that the 'β-D-1,4-acetamidodeoxyglucosidase' activity was unaffected. The inhibitory action of pentane was competitive and independent of pH, but was

[266] J. H. Hash, *Mol. Cell. Biochem.*, 1973, **2**, 103.
[267] 'Lysozyme', ed. E. F. Osserman, R. E. Canfield, and S. Beychok, Academic Press, New York, 1974.
[268] A. Grov, *Acta Chem. Scand.*, 1973, **27**, 2248.
[269] K. E. Hopper and H. A. McKenzie, *Mol. Cell. Biochem.*, 1974, **3**, 93.
[270] R. S. Mulvey, R. J. Gualtieri, and S. Beychok, *Biochemistry*, 1974, **13**, 782.
[271] S. K. Banerjee, I. Kregar, V. Turk, and J. A. Rupley, *J. Biol. Chem.*, 1973, **248**, 4786.
[272] K. Watanabe and S. Takesue, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1477.

dependent on the buffer strength. Spectral studies showed that the interaction with pentane affected the conformation of lysozyme, and the mechanism of the inhibition was discussed.

Since the amino-acid sequence of bovine α-lactalbumin has been shown to be remarkably similar to that of hen egg-white lysozyme, a procedure for refining the *X*-ray co-ordinates of proteins has been adapted to compute a low-energy conformation for α-lactalbumin; the structure obtained is similar to that of lysozyme.[273] A third refinement of the *X*-ray structure of lysozyme, by complete energy minimization, has been reported.[274] The final structure exhibited a very low conformational energy, yet maintained a close resemblance to the original *X*-ray structure. The optimized conformation was discussed in terms of the size of various energy contributions.

A reversible unfolding of the lysozyme molecule has been studied by means of the temperature-jump method and by measuring the change of absorbance of the pH-indicator 4-nitrophenol.[275] In the presence of lithium bromide, three relaxation processes were found, the fastest arising from a proton transfer between 4-nitrophenol and histidine-15. Only the slowest process was first-order, and was attributed to an unfolding of lysozyme. The results also indicated that the 'all-or-none' model can be used as a good approximation for the unfolding reaction. Reduced and disordered hen egg-white lysozyme rapidly regained enzymic activity in a non-enzymic system, and, at high temperatures, the native enzyme was formed as a transient intermediate.[276] That thermal inactivation did not depend on irreversible changes in the protein was shown by spontaneous activation of thermally inactivated lysozyme at 37 °C. The results are inconsistent with thermodynamic dictation of the protein structure.

N.m.r. measurements of the lattice relaxation of water in lysozyme crystals showed that the linewidths for heterogeneous systems must be interpreted with care, since the linewidth and its saturation behaviour are neither necessarily interpretable in terms of a single relaxation time nor related in a simple way to molecular motion.[277]

Reduction and alkylation of the disulphide bonds of hen egg-white lysozyme gave a material that did not cross-react immunologically with the intact molecule at the humoral level.[278] However, anti-lysozyme (induced with the aid of Freund's adjuvant) reacted with the denatured lysozyme. Since this reaction could not be inhibited by lysozyme, it was suggested that the antibodies involved arise in response to denatured forms in the immunogen, which, in turn, arise during emulsification in the adjuvant.

Isotherms for the adsorption of hen egg-white lysozyme on to chitin have been determined at various temperatures, and the initial rate of hydrolysis of chitin was shown to be proportional to the total concentration of the enzyme.[239] A study of the interaction between lysozyme and ovalbumin by sedimentation

[273] P. K. Warme, F. A. Momany, S. V. Rumball, R. W. Tuttle, and H. A. Scheraga, *Biochemistry*, 1974, **13**, 768.
[274] P. K. Warme and H. A. Scheraga, *Biochemistry*, 1974, **13**, 757.
[275] S.-I. Segawa, Y. Husimi, and A. Wada, *Biopolymers*, 1973, **12**, 2521.
[276] D. Wetlaufer, E. Kwok, W. L. Anderson, and E. R. Johnson, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 380.
[277] J. E. Jentoft and R. G. Bryant, *J. Amer. Chem. Soc.*, 1974, **96**, 297.
[278] R. J. Scibienski, *J. Immunol.*, 1973, **111**, 114.

equilibrium showed that a 1 : 1 complex predominates at low concentrations, but that higher-order complexes form as the concentration is increased.[279]

From an investigation of the antagonistic effects of amino-acids on the activity of lysozyme, it was evident that the enzyme is activated by L-glutamine and is inhibited by L-histamine.[280] The intestinal adsorption of ^{131}I-labelled hen egg-white lysozyme by rats has been investigated.[281] Trachoma-inclusion conjunctivitis induced by *Chlamydia trachomatis* can be reduced by lysozyme *in vitro*.[282]

The enzymic properties of lysozymes from *Nephthys hombergii* and hen egg-white have been compared; it was concluded that the enzymes could be differentiated by their affinities for *Micrococcus luteus* cells, by their responses to inhibition by 2-acetamido-2-deoxy-D-glucose, and by their actions on colloidal chitin and chito-oligosaccharides.[283]

A commercial preparation of crude β-amylase from barley contained an *endo-N*-acetylmuramidase (pI 9.5) active against cell walls of *Micrococcus lysodeikticus*.[284] The purified enzyme showed limited activity against intact cell walls, but hydrolysed the glycans of various bacterial cell walls from which non-peptidoglycan components had been partly removed. Extensive isoelectric focusing of the enzyme failed to remove all the β-amylase activity.[285] The susceptibility of the enzyme to various conditions and reagents was examined.

The susceptibilities of various strains of yeast to a cell-wall lytic enzyme produced by *Arthrobacter luteus*, and the effects thereon of treatments with heat, 2-thioethanol, or sodium dodecyl sulphate have been examined.[284]

Culture filtrates of *Bacillus subtilis* rapidly lysed cells of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*.[285] Since the lytic activity decreased on dialysis, a co-operating factor is assumed to participate in the lytic reaction, and a heat-stable, methanol-soluble, peptide-like factor was subsequently isolated and purified. Purification of the lytic preparation by ion-exchange chromatography gave three fractions, one of which hydrolysed the peptidoglycan from *P. aeruginosa* with the release of reducing groups but not of amino-groups. A homogeneous form of the enzyme hydrolysed the cell walls of *P. aeruginosa* only when the co-operative factor was added. It was concluded that the enzyme is a bacterial lysozyme capable of hydrolysing *N*-acetylmuramyl → 2-acetamido-2-deoxy-D-glucose linkages in peptidoglycans.

The lysozyme from bacteriophage T4 crystallizes in the space group $P3_121$ (or the enantiomorph) with the cell dimensions $a = b = 61.1$ Å and $c = 96.3$ Å.[286] Assuming a molecular weight of 1.87×10^4, there are six molecules per unit cell.

'Lysozymes' induced by various bacteriophages have been identified as *N*-acetylmuramic acid-L-alanine amidases.[287–289]

[279] G. J. Howlett and L. W. Nichol, *J. Biol. Chem.*, 1973, **248**, 619.
[280] R. V. Krishnamoorthy and E. Radha, *Enzyme*, 1974, **18**, 253.
[281] T. Yuzuriha, K. Katayama, and T. Fujita, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2807.
[282] L. Kondo, L. Hanna, and H. Keshishyan, *Proc. Soc. Exptl. Biol. Med.*, 1973, **142**, 131.
[283] J. P. Périn and P. Jollès, *Mol. Cell. Biochem.*, 1973, **2**, 189.
[284] T. Kaneko, K. Kitamura, and Y. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2295.
[285] S. Murao and Y. Takahara, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2671.
[286] B. W. Matthews, F. W. Dahlqvist, and A. Y. Maynard, *J. Mol. Biol.*, 1973, **78**, 575.
[287] M. Hongo, Y. Tahara, and S. Ogata, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 755.
[288] S. Ogata, Y. Tahara, and M. Hongo, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 763.
[289] M. Inouye, N. Arnheim, and R. Sternglanz, *J. Biol. Chem.*, 1973, **248**, 7247.

Analysis of the immune response in mice towards the loop region of lysozyme attached to a multi-chain poly-(DL-alanine) showed that the production of antibodies to the loop is genetically controlled by a unigenic dominant trait, which is not linked to the major histocompatability locus H-2.[290] Lysozyme has been immobilized by covalent attachment to polystyrene; the active complex was prepared so as to allow solubilization in non-aqueous media.[291]

Mannanases (Miscellaneous)

Mannanases have been found among the complex of polysaccharide hydrolases synthesized by thermotolerant *Actinomyces griseinus*; changes in the composition of the growth medium stimulating the formation of the mannanases concomitantly inhibited that of the glucanases.[251]

Oligo-1,6-D-glucosidases

The size (molecular weight 2.2×10^5) and number of subunits of the β-fructofuranosidase–oligo-1,6-glucosidase complex present in rabbit small intestines have been determined by gel filtration, fingerprint analysis of tryptic hydrolysates, and polyacrylamide gel electrophoresis in sodium dodecyl sulphate.[73] Of the two subunits (molecular weights $1.1—1.2 \times 10^5$) detected, one carries the β-D-fructofuranosidase activity and the other the oligo-1,6-D-glucosidase activity. The oligo-1,6-D-glucosidase (molecular weight 10^5) contains galactose (6), glucose (3), mannose (6), fucose (5), 2-amino-2-deoxygalactose (4), and 2-amino-2-deoxyglucose (21 units).

Pectate and Pectin Lyases

Pectin lyases from an *Arthrobacter* species and from 'Ultrazyme' have been investigated.[292] The best substrate for the purified, fungal pectin lyase was completely esterified pectin, whereas the best substrate for pectate lyases was 21—44%-esterified pectin. In view of reports in the literature of enzymes intermediate between the two classes, the activity of the enzymes towards pectate glycol ester was introduced as a new means for distinguishing between them, since only pectate lyases are active towards this substrate. In the light of results on the specificities of these enzymes, their classification and that of related enzymes were discussed in detail.

Nutritional factors relating to the synthesis of pectate lyases by strains of *Bacillus subtilis* and *Flavobacterium pectinovorum* have been examined in detail; the enzyme in the former source is constitutive, whereas that in the latter source is produced in quantity only if pectic substances are present.[293]

The synthesis of pectin lyase in *Fusarium oxysporum* and *Verticillium albo-atrum* has been investigated in the presence of restricted supplies of cell-wall sugars; D-galacturonic acid was particularly effective in inducing the synthesis of the enzyme under these conditions.[125]

[290] E. Maron, H. I. Scher, E. Mozes, R. Arnon, and M. Sela, *J. Immunol.*, 1973, **111**, 101.
[291] G. J. Bartling, H. D. Brown, and S. K. Chattopadhyay, *Biotechnol. Bioeng.*, 1974, **16**, 361.
[292] W. Pilnik, F. M. Rombouts, and A. G. J. Voragen, *Chem. Mikrobiol. Technol. Lebensm.*, 1973, **2**, 122.
[293] O. P. Ward and W. M. Fogarty, *Appl. Microbiol.*, 1974, **27**, 346.

Polygalacturonases

Polygalacturonase activity has been found among the digestive enzymes in the salivary gland and midgut of *Stenotus binotatus*, and the distribution of the enzyme was compared with that in other heteropterous insects.[111]

The action pattern and kinetics of an extracellular polygalacturonase from *Aspergillus niger* have been studied using oligosaccharides of D-galacturonic acid and terminal-reduced derivatives thereof.[294] The rates of hydrolysis decreased with shortening of the oligosaccharide chain; the disaccharide was not hydrolysed, whereas a productive complex was formed with the tetrasaccharide, resulting in the formation of mono- and tri-saccharides. Two and three modes of cleavage were observed with the penta- and hexa-saccharides, respectively. The enzyme was competitively inhibited by the trisaccharide, but not by the disaccharide. The reduced pentasaccharide formed a productive complex that furnished the reduced disaccharide and a reducing trisaccharide, but reduced oligosaccharides smaller than that from the pentasaccharide were not hydrolysed. It was concluded that the binding site of the polygalacturonase contains four subsites, and that the catalytic groups of the enzyme are located near the first glycosidic bond from the reducing end of the segment of the substrate bound in the complex.

The synthesis of polygalacturonase in *Fusarium oxysporum* and *Verticillium albo-atrum* by restricting the supplies of a number of cell-wall sugars has been investigated; D-galacturonic acid was particularly effective in inducing the synthesis of polygalacturonase under these conditions.[125]

The *endo*-polygalacturonase secreted by *Rhizopus stolonifer* infecting strawberries has been extracted with solutions of sodium chloride, after which it was partly purified by gel filtration and ion-exchange chromatography.[295] The enzyme (pH optimum 4.6—4.8) exhibited maximum stability at pH 4.0—6.0, and was inhibited by a number of reagents, including Hg^{2+} ions. The thermal stability of the enzyme was studied in detail, and sedimentation and diffusion data suggested that the molecule (molecular weight 6.0×10^4) has a high degree of asymmetry.

Isoenzymes of polygalacturonase (pI 5.5 and 9.7) have been separated from culture filtrates of *Sclerotinia fructigena* by isoelectric focusing and gel filtration.[70] Immunological studies were carried out with antibodies raised to the isoenzymes.

An active immobilized form of polygalacturonase has been prepared by coupling to a cyclic imidocarbonate derivative of macroporous agarose; the immobilized enzyme was used to obtain oligosaccharides comprising D-galacturonic acid.[296]

exo-Polygalacturonases

An *exo*-polygalacturonase has been purified from extracts of the mycelia of an *Acrocylindrium* species by ion-exchange chromatography.[297] The enzyme (pH optimum 4.5) possessed a higher affinity for poly(D-galacturonic acid) than for oligosaccharides derived therefrom. The enzyme hydrolysed the glycosidic

[294] Ľ. Rexová-Benková, *European J. Biochem.*, 1973, **39**, 109.
[295] A. S. Trescott and J. Tampion, *J. Gen. Microbiol.*, 1974, **80**, 401.
[296] F. E. A. van Houdenhoven, P. J. G. M. de Wit, and J. Visser, *Carbohydrate Res.*, 1974, **34**, 233.
[297] H. Kimura and S. Mizushima, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2589.

linkages of poly(D-galacturonic acid) from the non-reducing end, liberating D-galacturonic acid only.

In a study of the relation between the polygalacturonase activity and abscission in leaf explants of the citrus orange (*Citrus sinensis*), the enzymic activity could not be determined until extracts of the leaves were concentrated and an oxidase, which oxidized the reaction products, also present was inhibited.[298] The enzyme yielded D-galacturonic acid only from sodium polypectate, indicating that it is an *exo*-polygalacturonase. The increase in *exo*-polygalacturonase activity during abscission suggested that the enzyme participates in this process.

Pullulanases

Intracellular pullulanase has been extracted completely with sodium dodecyl sulphate from cells of *Aerobacter aerogenes*; after purification to homogenity (by ammonium sulphate fractionation, ion-exchange chromatography, and gel filtration), the enzyme (6.29*S*) was crystallized.[299] Polyacrylamide gel electrophoresis gave a main band and a sub-band (molecular weights 9×10^4 and 8×10^4, respectively), both of which were active. The extracellular pullulanase (molecular weight $6—7 \times 10^4$) is a slightly smaller molecule.

αα-Trehalases

The localization of disaccharidases, including αα-trehalase, in canine kidneys has been determined by a multiple-indicator dilution technique.[72] αα-Trehalase activity was shown to occur along the brush border of the proximal tube, where it is located near the transport receptors for D-glucose. Studies on the transport of D-glucose from disaccharides in hamster small intestines showed that the transport system is related to the disaccharidases, including αα-trehalase, present.[74]

The effects of storage on the αα-trehalase activities present in powders prepared from the termite *Trinervitermes trinervoides* have been investigated.[112] αα-Trehalase has been isolated from adult blowflies (*Calliphora erythrocephala*); the enzyme (pH optimum 5.5—6.3, K_m 3.2 m mol l^{-1}) exhibited maximum activity at 45—55 °C and is specific for αα-trehalose.[300]

Xylanases (Miscellaneous)

A preparation of cellulase from workers of the termite *Trinervitermes trinervoides* was also able to hydrolyse β-1,4-xylan, but the two activities could not be separated by electrophoresis.[225]

In a study of the synthesis of cell-wall-degrading enzymes in vascular wilt fungi caused by restricted supplies of sugars, D-xylose and, to a lesser extent, D-galacturonic acid were effective in inducing xylanase activity.[125]

Carbohydrate Epimerases

Poly(D-mannuronic Acid) 5-Epimerases.—An enzyme that catalysed the conversion of poly(D-mannuronic acid) into a polysaccharide also containing

[298] J. Riov, *Plant Physiol.*, 1974, **53**, 312.
[299] R. Ohba and S. Ueda, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2821.
[300] H. Duve, *Insect Biochem.*, 1973, **2**, 445.

L-guluronic acid residues has been partially purified from the marine brown alga *Pelvetia canaliculata*.[301] The enzymic activity was measured by changes in the response of the substrate in the carbazole reaction and from the incorporation of tritium into the polysaccharide produced. The enzyme appeared to be a poly-(D-mannuronic acid) 5-epimerase.

Carbohydrate Isomerases

D-Arabinose Isomerases.—D-Arabinose (L-fucose) isomerase has been obtained from extracts of the cells of *Aerobacter aerogenes* grown on D-arabinose by repeated fractional precipitation with poly(ethylene glycol).[302] The crystalline enzyme (15.4*S*, molecular weight 2.5×10^5 by gel filtration) was homogeneous by ultracentrifugation and by polyacrylamide gel electrophoresis. The pH optima for D-arabinose and L-fucose isomerases were identical (pH 9.3, K_m 51 and 160 mmol l^{-1}, respectively), and both activities were inhibited by the pentitols (K_i being constant for a particular inhibitor). It was concluded that a single enzyme species is responsible for both activities.

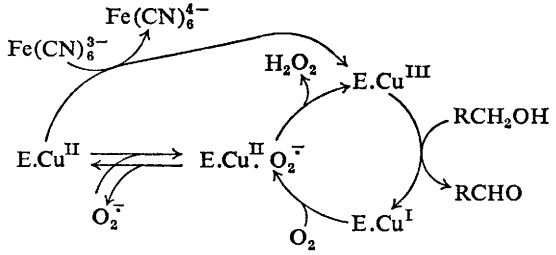
D-Glucose Isomerases.—The rates of formation of D-glucose isomerase by a *Streptomyces* species grown on various media have been determined; the best results were obtained with xylobiose.[303] However, the concentration of D-glucose isomerase was low compared with that produced by other organisms.

Carbohydrate Oxidases

D-Galactose Oxidase.—In a study of the specificity of D-galactose oxidase, glycoproteins containing terminal, non-reducing D-galactofuranosyl residues were treated with the enzyme and then reduced with borotritide ion.[304] On degradation of the products with periodate ion, the label was isolated in the form of [^{3}H]formaldehyde, confirming that the enzyme oxidized terminal, non-reducing D-galacto-furanosyl and -pyranosyl units at C-6.

Evidence for the involvement of copper(III) in the enzymic reaction of D-galactose oxidase has been reported.[305] Superoxide dismutase reduced the rate of the reactions catalysed by D-galactose oxidase and caused a small increase in the e.s.r. signal attributed to copper(II). Both ferricyanide and superoxide activated the enzyme and produced a decrease in the e.s.r. signal for copper(II). These and other results suggested the mechanism shown in Scheme 5. In this cycle, the enzyme oscillates between copper(I) and copper(III) forms, with superoxide bound to the copper(II) form representing a transient intermediate. The copper(III)-enzyme apparently converts the primary hydroxy-group of D-galactose into an aldehyde. Superoxide dissociates from the copper(II) intermediate once in several hundred or so cycles to give a copper(II)-enzyme, which is catalytically inactive. Only when it reacts with a one-electron reagent can the copper(II)-enzyme re-enter the cycle.

[301] J. Madgwick, A. Haug, and B. Larsen, *Acta Chem. Scand.*, 1973, **27**, 3592.
[302] K. Izumori and K. Yamanaka, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 267.
[303] Y. Takasaki, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 667.
[304] R. J. Sturgeon, *L'Actualité chimique*, 1974, No. 7, 41.
[305] G. A. Hamilton, R. D. Libby, and C. R. Hartzell, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 333.



E = enzyme.
R = rest of D-galactosyl residue.

Scheme 5

NN'-Ethylenebis(trifluoroacetylacetoniminate)copper(II) has been proposed as a model for the equatorial co-ordination of copper(II) in D-galactose oxidase.[306] Differences between the Hamiltonian spin parameters of the two complexes were ascribed to the presence of a strongly π-bonding, axial ligand in the enzyme. Calculations of the *d–d* electronic transitions of copper(II) in both the model and the enzyme, based on observed e.s.r. parameters, were in good agreement with the optical spectra. Possible features of the enzyme–copper(II) complex were discussed. The addition of potential copper(II) ligands to solutions of D-galactose oxidase produced distinctive changes in the e.s.r. spectrum; such strongly bound ligands as cyanide ion were capable of forming π-bonds to Cu^{II}.[307] The substrate (D-galactose) competed with this ligand for the copper(II) site, whereas the product (hydrogen peroxide) appeared to bind at copper(II), although the effect on the e.s.r. spectrum was small. It was concluded that only a single co-ordination site in the complex is readily accessible to exogenous ligands, and that this site is normally occupied by a water molecule or hydroxide ion.

C.d. spectra have been recorded for D-galactose oxidase from *Polyporus circinatus* in the presence of its substrates and products in an attempt to ascertain which of them interacts with the copper atom of the enzyme.[308] The results suggested that, during the course of the enzymic reaction, oxygen binds after D-galactose. The bound oxygen interacts directly with (potential) reducing groups on the substrate, rather than within the inner co-ordination sphere of the copper atom. The product of the enzymic reaction, *galacto*-hexodialdose, interacted with the copper atom in a manner similar to D-galactose. Large changes in the u.v. and c.d. spectra brought about by dihydroxyacetone and *galacto*-hexodialdose indicated that an L-tryptophanyl residue is located at or near the active site.

D-Glucose Oxidases.—It has been shown that the reaction of D-glucose with D-glucose oxidase can be monitored for the production of D-gluconic acid by differential conductivity without prior saturation of the substrate with oxygen.[309] To ensure an adequate supply of oxygen for the reaction, a catalase impurity in

[306] R. S. Giordano and R. D. Bereman, *J. Amer. Chem. Soc.*, 1974, **96**, 1019.
[307] R. S. Giordano, R. D. Bereman, D. J. Kosman, and M. J. Ettinger, *J. Amer. Chem. Soc.*, 1974, **96**, 1023.
[308] M. J. Ettinger and D. J. Kosman, *Biochemistry*, 1974, **13**, 1247.
[309] R. A. Messing, *Biotechnol. Bioeng.*, 1974, **16**, 525.

the enzyme preparation was used to recycle oxygen; hydrogen peroxide was also added to the solution to ensure an adequate supply of oxygen initially.

D-Glucose oxidase obtained from *Aspergillus niger* comprises 10.5% of carbohydrate, according to the reaction with phenol–sulphuric acid, consisting of galactose (16—17%), glucose (0.6%), mannose (75—76%), 2-amino-2-deoxygalactose (5—6%), and 2-amino-2-deoxyglucose (2%).[310] Periodate oxidation of the enzyme did not alter the protein conformation, catalytic properties, or thermal stability. That the thermal stability was reduced in the presence of sodium dodecyl sulphate was evident from a marked decrease in the entropy of activation. It was concluded that the carbohydrate portion of the molecule contributes to the stability, but does not affect the overall structure.

Reconstitution of flavin adenine dinucleotide (FAD) and the apoenzyme of D-glucose oxidase from *A. niger* was strongly inhibited by 4-chloromercuribenzoate, although the enzyme's activity *per se* was not inhibited.[311] This inhibition was reversed by dithiothreitol. Loss of sigmoidicity was observed in the 'concentration–activity' curve of FAD in the reconstitution process when the apoenzyme was incubated with FAD in the presence of the inhibitor. Adenosine and analogues thereof having the amino-group at C-6 of the purine base replaced by a thiol group showed strong inhibition of the incorporation of FAD in the reconstitution process. In the reconstitution process initiated by flavin hypoxanthine dinucleotide, prolonged incubation with, and a high concentration of, the dinucleotide were necessary to regenerate the activity, due to the lack of a binding site initially.

D-Glucose oxidase has been used in conjunction with an oxygen electrode in an improved assay of mutarotase.[312]

Active immobilized forms of D-glucose oxidase have been obtained by covalent attachment to a derivative of glass,[313] by entrapment in a cross-linked gel of 2-hydroxyethyl methacrylate containing 20% of poly(vinyl pyrrolidone),[314] and by electrodeposition with collagen on helical supports of stainless steel.[315] The enzyme together with peroxidase have been immobilized by physical entrapment in polymeric fibres [79] and in polyacrylamide polymerized on nylon netting,[316] and by covalent attachment to a diazo-derivative of a poly(acrylic acid).[316] D-Glucose oxidase together with β-fructofuranosidase and catalase have also been immobilized by physical entrapment in polymeric fibres.[79]

Glycopeptide Linkage Hydrolases

***N*-Acetylmuramyl-L-alanine Amidases.**—A lytic enzyme isolated from a phage-induced lysate of *Clostridium saccharoperbutylacetonicum* has been purified to homogeneity by gel electrophoresis. The enzyme (pH optimum 6.5, temperature optimum 30—35 °C, pI 4.0), which was indicated to have a molecular weight

[310] S. Nakamura and S. Hayashi, *F.E.B.S. Letters*, 1974, **41**, 327.
[311] H. Tsuge and H. Mitsuda, *J. Biochem.* (*Japan*), 1974, **75**, 399.
[312] I. Miwa and J. Okuda, *J. Biochem.* (*Japan*), 1974, **75**, 1177.
[313] M. K. Weibel, W. Dritschilo, H. J. Bright, and A. E. Humphrey, *Analyt. Biochem.*, 1973, **52**, 402.
[314] I. Hinberg, A. Kapoulas, R. Korus, and K. O'Driscoll, *Biotechnol. Bioeng.*, 1974, **16**, 159.
[315] A. Constantinides, W. R. Vieth, and P. M. Fernandes, *Mol. Cell. Biochem.*, 1973, **1**, 127.
[316] G. Nagy, L. H. von Storp, and G. G. Guilbault, *Analyt. Chim. Acta*, 1973, **66**, 443.

of 10^4 by polyacrylamide gel electrophoresis in sodium dodecyl sulphate, was stimulated by H_4edta.[287] The specificity of the enzyme was restricted to certain *Clostridium* species. The action of this phage-induced lytic enzyme on the cell-wall peptidoglycan of *C. saccharoperbutylacetonicum* was studied in detail; digestion of the cell wall was accompanied by the release of *N*-terminal L-alanine residues without concomitant release of *C*-terminal amino-acids or reducing groups.[288] It was concluded that the enzyme cleaves the linkage between *N*-acetylmuramic acid and L-alanine, and is an *N*-acetylmuramyl-L-alanine amidase.

The specificity of a lytic enzyme induced by infecting *Escherichia coli* with bacteriophage T7 has been investigated by analysing the products of the enzyme's action on a purified peptidoglycan from the same organism.[289] When a radio-labelled peptidoglycan was digested with bacteriophage T4 lysozyme, lipoprotein, shown to have a molecular weight of 1.06×10^4 by polyacrylamide gel electrophoresis, was cleaved from the peptidoglycan. The lipoprotein was shown to contain labelled 2-acetamido-2-deoxy-D-glucose and diaminopimelic acid. However, the T7-enzyme gave a product of lower molecular weight that did not contain 2-acetamido-2-deoxy-D-glucose and, therefore, is not derived from the glycan backbone, but from the peptide region. On the basis of these and other results, it was concluded that the T7-enzyme cleaves the *N*-acetylmuramic acid—L-alanine bond and is a glycopeptidase. It is incorrect to refer to the T7-enzyme as lysozyme.

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases.—Extracts from human, boar, ram, and squirrel-monkey spermatozoa and a human acrosomal extract have been shown to contain 4-L-aspartyl-β-D-glucosylamine amidohydrolase activity.[317] The enzyme was purified by chromatography on phosphocellulose and gel filtration.

β-D-Xylosyl-L-serine Glycopeptidases.—In the scanning of numerous organisms for β-D-xylosidase activity, *Aspergillus niger* and *Penicillium wortmanni* have been examined for the effects of inducers and surfactants on the yield of the enzyme, which also hydrolysed β-D-xylopyranosyl-L-serine.[183]

Proteinases

Aminopeptidases.—Prolonged incubation of a particulate aminopeptidase (microsomal) from porcine kidney with neuraminidase, α-D-mannosidase, and β-D-acetamidodeoxyglucosidase released virtually all of the sialic acid, more than 60% of the neutral sugars, and 45% of the 2-acetamido-2-deoxy-D-glucose originally bound to the enzyme.[318] Although the modified enzyme differed from the natural form in its electrophoretic mobility and solubility, the catalytic activity, substrate specificity, and the inhibition by H_4edta and 1,10-phenanthroline were the same. It was concluded that the carbohydrate moieties do not contribute to the activity of the enzyme *in vitro*.

Ficins.—Ficin from *Ficus carica*, purified to homogeneity by electrophoresis and ion-exchange chromatography, has been crystallized.[319] The enzyme

[317] V. K. Bhalla, W. L. Tillman, and W. L. Williams, *J. Reprod. Fert.*, 1973, **34**, 137.
[318] H. Wacker, *Biochim. Biophys. Acta*, 1974, **334**, 417.
[319] M. Sugiura and M. Sasaki, *Biochim. Biophys. Acta*, 1974, **350**, 38.

comprises neutral carbohydrate (4.8%), which did not dissociate from the protein during a number of separation procedures. The enzyme (molecular weight 2.6×10^4, pH optimum 8.0, temperature optimum 60 °C, pI 9.1) was stable over a broad pH range, was activated by L-cysteine and 2-thioethanol, but was inhibited by mercuric chloride and 4-chloromercuribenzoate. Ficin S was shown to differ from ficins A—D only in pI value and in the content of carbohydrate.

Ficin has been purified by affinity chromatography on a cyclic imidocarbonate derivative of agarose to which 4-aminophenylmercuric acetate had been coupled; this procedure separated the enzyme into fractions containing ficin and mercurificin.[320]

Thrombins.—Concanavalin A has been found to prolong the clotting time of human plasma and purified fibrinogen by thrombin, due to a delay in the release of peptides from fibrinogen.[321] The rate of fibrin-monomer polymerization was not affected. The lectin inhibited the hydrolysis of protamine sulphate by thrombin, but all these effects were prevented by methyl α-D-mannopyranoside. From these and other results, it was concluded that concanavalin A binds to the carbohydrate moiety of thrombin, thereby inhibiting the enzymic activity.

Ribonucleases

Giraffe pancreatic ribonuclease I has been purified by precipitation with ammonium sulphate and affinity chromatography on an agarose derivative to which 5′-(4-aminophenyl)uridine 2′3′-cyclic phosphate had been coupled.[322] The amino-acid sequence of the enzyme, which also contains D-galactose, D-mannose, L-fucose, 2-acetamido-2-deoxy-D-glucose, and 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid, was determined.

Miscellaneous Enzymes

L-Aspartate Aminotransferases.—A cytoplasmic L-aspartate aminotransferase obtained from porcine heart is associated with carbohydrate comprising D-galactose, D-glucose, D-mannose, D-xylose, L-rhamnose, acidic sugars, and 2-amino-2-deoxyhexose.[323] Hydrolytic studies indicated that the carbohydrate is bound to the protein by *O*-glycosidic linkages to L-serine and/or L-threonine residues. Further fractionation of the aspartate aminotransferase was achieved by ion-exchange chromatography; the carbohydrate content of the fractions differed and was related to the mobility of the fraction towards the anode.

Ceruloplasmins.—The substrate specificity of ceruloplasmin has been studied, particularly with phenylalkylamines as substrates.[324]

Chitin Deacetylases.—An enzyme that hydrolyses the *N*-acetyl groups of the 2-acetamido-2-deoxy-D-glucosyl units of chitin has been partially purified from *Mucor rouxii* by ion-exchange chromatography.[325] The enzyme also deacetylated

[320] C. D. Anderson and P. L. Hall, *Analyt. Biochem.*, 1974, **60**, 417.
[321] S. Karpatkin and M. Karpatkin, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1111.
[322] W Gaastra, G. Groen, G. W. Welling, and J. J. Beintema, *F.E.B.S. Letters*, 1974, **41**, 227.
[323] G. F. Denisova and O. L. Polyanovskii, *Biokhimiya*, 1974, **39**, 401.
[324] B. C. Barrass, D. B. Coult, P. Rich, and K. J. Tutt, *Biochem. Pharmacol.*, 1974, **23**, 47.
[325] Y. Araki and E. Ito, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 669.

N-acetylchito-oligosaccharides, but it was inactive toward bacterial cell-wall peptidoglycan, *N*-acetylated heparin, a polymer of 2-acetamido-2-deoxy-D-galactose, di-*N*-acetylchitobiose, and 2-acetamido-2-deoxy-D-glucose. The enzyme (pH optimum 5.5), whose presence accounts for the formation of chitosan in fungi, was inhibited by acetate ions.

Dopamine β-Mono-oxygenases.—Concanavalin A and dopamine β-mono-oxygenase from the chromaffin vesicles of bovine adrenal glands formed a complex that could be dissociated quantitatively with methyl α-D-mannopyranoside.[326] This procedure enabled the enzyme to be separated from other proteinaceous materials in the vesicles, and a highly pure preparation was obtained after a single passage through a column containing the immobilized lectin. It was concluded that the enzyme contains a highly branched carbohydrate portion having α-D-mannopyranosyl and/or α-D-glucopyranosyl residues as non-reducing termini.

Glycogen (Starch) Synthetases.—A glycogen (starch) synthetase present in homogenates of human polymorphonuclear leucocytes has been purified to high specific activity by affinity chromatography on immobilized concanavalin A.[327] The conversion of this enzyme (D type) into the I type was studied, and the latter enzyme was also purified in a similar way. From the interaction of each with concanavalin A, it was assumed that both types are glycoproteins.

Indole-3-acetic Acid Oxidases.—Fractions from pea (*Pisum sativum*) roots containing indole-3-acetic acid oxidase have been separated into glycoprotein and protein forms by affinity chromatography on immobilized concanavalin A.[328] The development of the activity, which was inseparable from peroxidase activity, in the roots was investigated in detail.

α-Lactalbumins.—The preparation of α-lactalbumin from human, ovine, porcine, and other mammalian milk has been reported.[269] α-Lactalbumin is absent from the milk of several species. The amino-acid compositions of α-lactalbumins and lysozymes from a number of species were compared.

A computed structure for bovine α-lactalbumin resembled the *X*-ray structure of lysozyme (see also p. 374).[273]

α-Lactalbumin has been immobilized by covalent attachment to an agarose derivative.[329]

Lactose Synthetases.—A review of lactose synthetases has covered the requirement for both D-galactosyltransferase activity and α-lactalbumin, and the purification, catalytic properties, and biological significance of D-galactosyltransferase.[330]

A D-galactosyltransferase has been isolated from cow's milk; the existence of two forms (molecular weights $4.2—4.4 \times 10^4$ and $5.5—5.9 \times 10^4$) was demonstrated by polyacrylamide gel electrophoresis and gel filtration.[328] The forms were similar with respect to their catalytic properties, inhibition by heat

[326] R. A. Rush, P. E. Thomas, S. H. Kindler, and S. Udenfriend, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1301.
[327] H. Sølling and P. Wang, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 1234.
[328] B. Darbyshire, *Physiol. Plantarum*, 1973, **29**, 293.
[329] S. C. Magee, R. Mawal, and K. E. Ebner, *Biochemistry*, 1974, **13**, 99.
[330] K. E. Ebner, in 'The Enzymes', 'Group Transfer, Part B', ed. P. D. Boyer, Academic Press, New York, 1973, 3rd edn., Vol. 9, p. 363.

and by sulphhydryl compounds, and retention on immobilized α-lactalbumin. Both forms contain carbohydrate, but two bands still appeared on electrophoresis after desialylization.

The presence of lactose synthetase in milk samples from placental and pouch-bearing mammals has been investigated.[269] The preparation of lactose synthetase and investigations of its properties were described in several cases.

Levansucrases.—An inducible exocellular activity of *Bacillus subtilis* has been shown to be a levansucrase; the purified enzyme (4.16*S*, molecular weight 4.0×10^4) consists of a single chain with an *N*-terminal L-lysine residue.[77] Kinetic aspects of the transfructosylation reaction catalysed by the enzyme were studied, and properties of the enzyme were compared with those of levansucrase obtained from another strain of the organism.

Peroxidases.—Fractions from pea (*Pisum sativum*) roots containing peroxidase and indole-3-acetic acid oxidase activities have been separated into glycoproteinaceous and other forms by affinity chromatography on immobilized concanavalin A.[328] The two activities could not be separated, and their development in pea roots was investigated.

Phosphatases.—Ovine-brain acid phosphatase has been found to bind with concanavalin A to form an enzymically active precipitate, indicating that the enzyme is a glycoprotein.[57] The activity of the complex was inhibited by methyl α-D-glucopyranoside, which also dissociated the complex. Formation of the complex could be used in purifying the enzyme.

Alkaline phosphatase has been purified from the microvillar membranes of porcine kidney.[331] The enzyme exists in discrete, multiple forms having different degrees of glycosylation of the polypeptide chains, which appear to be identical. Since the forms differed in stability, charge, molecular size, and catalytic and antigenic activities, they are unlikely to be isoenzymes.

Phosphodiesterases.—Since a venom phosphodiesterase I could be attached to an immobilized form of concanavalin A by direct interaction, it appears that the enzyme is a glycoprotein.[332]

Sulphatases.—Serum arylsulphatase activity has been measured for patients with arthritis and with other disorders.[47] The properties of the residual arylsulphatase B activity in cultured fibroblasts of cases of Maroteaux–Lamy syndrome were identical with those of the enzyme from controls.[333] Normalization of the decreased turnover of glycosaminoglycans in the fibroblasts of the patients, by supplementation with arylsulphatase B from normal fibroblasts, is consistent with the view that the primary biochemical defect in the syndrome is a deficiency of arylsulphatase B.

Two forms of arylsulphatase A have been isolated from human urine by reverse-gradient solubilization chromatography using ammonium sulphate.[334]

[331] E. D. Wachsmuth and K. Hiwada, *Experientia*, 1973, **29**, 760.
[332] E. Sulkowski and M. Laskowski, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 463.
[333] A. L. Fluharty, R. L. Stevens, D. L. Saunders, and H. Kihara, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 455.
[334] R. L. Stevens, M. Hartman, A. L. Fluharty, and H. Kihara, *Biochim. Biophys. Acta*, 1973, **302**, 338.

Both enzymes possessed similar activities towards 4-nitrocatechol sulphate, 4-methylumbelliferyl sulphate, and cerebroside sulphate; inactivation and immunological studies did not distinguish between them. The β-form partly reverted to the α-form on storage, and the effects of various reagents on the interconversion were studied.

A test for enzyme–antibody binding has been developed with a view to studying antigenic differences between normal and mutant enzymes that cannot be distinguished by routine immunoprecipitation techniques.[335] The test was applied to a mutant enzyme in metachromatic leukodystrophy using immobilized anti-(human arylsulphatase A). Assay of the mutant and homologous normal enzymes is based on their ability to saturate the bound antibody, thus competitively blocking any binding of a standard arylsulphatase. Enzymic activity was then determined using a nitrocatechol sulphate as substrate. Significant differences between the normal and mutant enzymes were revealed by this technique. The assay appeared to be generally applicable and was also used to compare human, monkey, and canine arylsulphatases A.

A preparation of bovine-brain arylsulphatase B (4.4*S*, molecular weight 6.0×10^4) was shown to be homogeneous by sedimentation at pH 5.0 and 7.5; physicochemical measurements showed that the molecule is globular.[336] However, ion-exchange chromatography yielded seven incompletely resolved fractions, whose components were analysed and compared with those of arylsulphatase A.

Ovine-brain arylsulphatase A has been found to bind to concanavalin A to form an enzymically active complex, indicating that the enzyme is a glycoprotein.[57] The activity of the complex was inhibited by methyl α-D-glucopyranoside, which also dissociated it (maximum dissociation at pH 9.0).

Arylsulphatases have been found in the digestive systems of 18 species of Echinoderms.[160] The A and B activities were located mainly in the pyloric aceca of Asteroids and in the oesophagus of Echinoids; the distribution of the activities was considered with respect to feeding habits.

Chondroitin sulphate sulphatase from embryonic chick cartilage has been shown to be less active towards chondroitin sulphate than towards oligosaccharides derived therefrom.[241] The enzyme was inhibited by citrate–phosphate buffer, and its functional relation to other endogenous enzymes active towards chondroitin sulphate was discussed.

Thio-D-glucosidases.—Sonicated extracts of crambe (*Crambe abyssinica*) seed meal, prepared in the presence of Fe^{2+} ion and dithiothreitol, converted *epi*-progoitrin into D-glucose, HSO_4^-, 1-cyano-2-hydroxy-3,4-epithiobutanes, and 1-cyano-2-hydroxybut-3-ene.[337] A fraction of the extract contained a 1-thio-D-glucosidase that converted *epi*-progoitrin into 1-cyano-2-hydroxybut-3-ene. A proteinaceous material which was also separated did not hydrolyse *epi*-progoitrin, but, in the presence of thio-D-glucosidase, promoted the formation of 1-cyano-2-hydroxy-3,4-epithiobutanes to the same extent as that by crude extracts of the seed meal.

[335] E. Neuwelt, P. F. Kohler, and J. Austin, *Immunochemistry*, 1973, **10**, 767.
[336] W. S. Bleszynski and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **317**, 164.
[337] H. L. Tookey, *Canad. J. Biochem.*, 1973, **51**, 1654.

The properties of thio-D-glucosidase isoenzymes from rape seed (*Brassica napus*), white mustard (*Sinapis alba*) seed, and *B. campestris* seed have been compared.[338] Although the isoenzymes varied considerably in physicochemical and enzymic properties, there was no significant difference in the substrate specificities.

An intracellular thio-D-glucosidase (pH optimum 6.2, temperature optimum 34 °C) produced by *Aspergillus niger* was stimulated by Cu^{+}, Cu^{2+}, Mn^{2+}, and Co^{2+} ions, but was inhibited by Hg^{2+} and Sn^{2+} ions; however, metal-complexing reagents had little effect.[339] In contrast to the plant enzyme, this thio-D-glycosidase was unaffected by L-ascorbic acid, and the relation between them was discussed.

Index of Enzymes Referred to in Chapter 6*

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
β-D-Acetamidodeoxy-galactosidase	2-acetamido-2-deoxy-β-D-galactoside acetamidodeoxygalactohydrolase	3.2.1.53	330
α-D-Acetamidodeoxy-glucosidase	2-acetamido-2-deoxy-α-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.50	330
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β-D-Acetamidodeoxy-glucosidase	2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	330
β-D-Acetamidodeoxy-hexosidase	2-acetamido-2-deoxy-β-hexoside acetamidodeoxyhexohydrolase	3.2.1.52	330
N-Acetylmuramyl-L-alanine amidase	mucopeptide amidohydrolase	3.5.1.28	381
Acid phosphatase	orthophosphoric monoester phospho-hydrolase (acid optimum)	3.1.3.2	385
Agarase	agarose 3-glycanohydrolase	3.2.1.81	358
Alginate lyase	poly(1,4-β-D-mannuronide) lyase	4.2.2.3	358
Alkaline phosphatase	orthophosphoric monoester phospho-hydrolase (alkaline optimum)	3.1.3.1	385
Aminopeptidase (microsomal)	α-aminoacyl-peptide hydrolase (microsomal)	3.4.11.2	382
α-Amylase	1,4-α-D-glucan glucanohydrolase	3.2.1.1	358
β-Amylase	1,4-α-D-glucan maltohydrolase	3.2.1.2	362
Arabinanase			363
α-L-Arabinofuranosidase	α-L-arabinofuranoside arabinohydrolase	3.2.1.55	337
D-Arabinose isomerase	D-arabinose ketol isomerase	5.3.1.3	379
Arylsulphatase	aryl sulphate sulphohydrolase	3.1.6.1	385
L-Aspartate aminotransferase	L-aspartate:2-oxoglutarate aminotransferase	2.6.1.1	383
4-L-Aspartyl-β-D-glucosyl-amine amidohydrolase	2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucosylamine	3.5.1.37	382
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Carbohydrate oxidases			379
Cellulase	1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase	3.2.1.4	363
Ceruloplasmin			383

[338] R. Björkman and B. Lönnerdal, *Biochim. Biophys. Acta*, 1973, **327**, 121.
[339] M. Ohtsuru and T. Hata, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2543.

* See Introduction (Part II, Chapter 1, p. 191).

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
Chitin deacetylase			383
Chitinase	poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)]glycanohydrolase	3.2.1.14	366
Chitosanase			366
Chondroitin sulphate hydrolase			366
Chondroitin sulphate sulphatase			385
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Dextranase	1,6-α-D-glucan 6-glucanohydrolase	3.2.1.11	367
Dopamine β-mono-oxygenase	3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating)	1.14.17.1	384
Ficin		3.4.22.3	382
β-D-Fructofuranosidase	β-D-fructofuranoside fructohydrolase	3.2.1.26	338
α-L-Fucosidase	α-L-fucoside fucohydrolase	3.2.1.51	339
β-D-Fucosidase†	β-D-fucoside fucohydrolase	3.2.1.38	339
Galactanase			368
D-Galactose oxidase	D-galactose:oxygen 6-oxidoreductase	1.1.3.9	379
α-D-Galactosidase	α-D-galactoside galactohydrolase	3.2.1.22	341
β-D-Galactosidase	β-D-galactoside galactohydrolase	3.2.1.23	341
β-D-Galactosylceramidase	D-galactosyl-*N*-acylsphingosine galactohydrolase	3.2.1.46	341
β-D-Galactosyl-D-galactosyl-D-glucosylceramidase	D-galactosyl-D-galactosyl-D-glucosyl-ceramide galactohydrolase	3.2.1.47	341
endo-β-1,3-Glucanase	1,3-β-D-glucan glucanohydrolase	3.2.1.39	369
endo-β-1,6-Glucanase	1,6-β-D-glucan glucanohydrolase	3.2.1.75	369
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D-Glucose oxidase	β-D-glucose:oxygen 1-oxidoreductase	1.1.3.4	380
α-D-Glucosidase	α-D-glucoside glucohydrolase	3.2.1.20	348
β-D-Glucosidase	β-D-glucoside glucohydrolase	3.2.1.21	348
exo-β-1,4-D-Glucosidase	1,4-β-D-glucan glucohydrolase	3.2.1.74	371
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Heparin lyase	heparin lyase	4.2.2.7	371
Hyaluronidase	hyaluronate 4-glycanohydrolase	3.2.1.35	371
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Keratan sulphate hydrolase			372
α-Lactalbumin	see	2.4.1.22	384

† This entry is now deleted from tables of Enzyme Nomenclature, but is retained in this Index and Report since it is 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*
Lactose synthetase	UDP-galactose:D-glucose 4-β-galactosyltransferase	2.4.1.22	384
Laminarinase	1,3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	3.2.1.6	372
Levansucrase	sucrose:2,6-β-D-fructan 6-β-fructosyltransferase	2.4.1.10	385
Lysozyme	mucopeptide *N*-acetylmuramyl-hydrolase	3.2.1.17	373
Mannanases (miscellaneous)			376
α-D-Mannosidase	α-D-mannoside mannohydrolase	3.2.1.24	354
β-D-Mannosidase	β-D-mannoside mannohydrolase	3.2.1.25	354
Neuraminidase	acylneuraminyl hydrolase	3.2.1.18	355
Oligo-1,6-D-glucosidase	dextrin 6-α-glucanohydrolase	3.2.1.10	376
Pectate lyase	poly(1,4-α-D-galacturonide) lyase	4.2.2.2	376
Pectin lyase	poly(methoxygalacturonide) lyase	4.2.2.10	376
Peroxidase	donor:hydrogen peroxide oxidoreductase	1.11.1.7	385
Phloretin-β-D-glucosidase	phlorizin glucohydrolase	3.2.1.62	348
Phosphatases			385
Phosphodiesterase I	oligonucleate 5′-nucleotidohydrolase	3.1.4.1	385
Polygalacturonase	poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15	377
exo-Polygalacturonase	poly(1,4-α-D-galacturonide) galacturonohydrolase	3.2.1.67	377
Poly(D-mannuronic acid) 5-epimerase			378
Proteinases			382
Pullulanase	pullulan 6-glucanohydrolase	3.2.1.41	378
Ribonuclease I	ribonucleate 3′-pyrimidino-oligonucleotidohydrolase	3.1.4.22	383
Sialidase‡			355
Sulphatase			385
D-Thioglucosidase	thioglucoside glucohydrolase	3.2.3.1	386
Thrombin		3.4.21.5	383
αα-Trehalase	αα-trehalose glucohydrolase	3.2.1.28	378
Xylanase (miscellaneous)			378
α-D-Xylosidase	α-D-xyloside xylohydrolase		357
β-D-Xylosidase	β-D-xyloside xylohydrolase	3.2.1.37	357
β-D-Xylosyl-L-serine glycopeptidase			382

‡ Sialidase is generally used to include neuraminidase and unspecified acylneuraminyl hydrolases.

7
Glycolipids and Gangliosides

BY R. J. STURGEON

Introduction

A comprehensive and critical survey of the role of lipids in the biosynthesis of glycans has been published.[1] The growth behaviour of transformed cells, as related to surface glycolipids and glycoproteins, has been reviewed.[2] Another review has covered the biosynthesis of glycolipids in virus-transformed cells.[3] Multiple forms of galactosidases, glucosidases, hexosaminidases, α-mannosidase, α-fucosidase, and α-iduronidase of human tissues have been surveyed, and their relations to known disorders in the metabolism of glycolipids, glycoproteins, and mucopolysaccharides have been discussed.[4] The structures, biogeneses, and functions of plant glycolipids have been reviewed.[5]

Fatty acids, fatty-acid aldehydes, phospholipids, glycolipids, and cholesterol have been stained with Malachite Green on t.l.c. plates.[6] Anomalous chromatographic properties of D-mannosyldolichol phosphate in the presence of microsomal lipids have been reported.[7] 4-Phenyl-spiro[furan-2(3*H*),1-phthalan]-3,3′-dione has been used in determinations of sphingosine bases and sphingolipids; amino-sugars also react with this reagent, but may be removed by solvent extraction.[8] An evaluation of g.l.c. methods for the quantitative determination of hexoses derived from neutral lipids has been reported.[9] Analytical procedures have been described for the determinations of amino-sugars, carbohydrates, fatty acids, and 3-deoxy-2-octulosonic acid in endotoxic glycolipids.[10] The detection of sialic-acid-containing gangliosides, after separation by polyacrylamide gel electrophoresis, has been achieved using a resorcinol reagent following periodate oxidation of the molecules.[11] The mass spectra of reduced derivatives of glycosphingolipids showed abundant ions containing the complete carbohydrate and fatty acid residues, allowing conclusions to be drawn concerning the ratios of hexoses to

[1] F. W. Hemming, in 'Biochemistry of Lipids', ed. T. W. Goodwin (MTP International Review of Science), Biochemistry Series 1, Butterworths, London, 1974, Vol. 4, p. 39.
[2] S. Hakomori, C. G. Gahmberg, R. A. Laine, and D. Kiehn, in 'Membrane Transformations in Neoplasma', ed. J. Schultz and R. E. Block, Academic Press, London and New York, 1974, p. 297.
[3] R. O. Brady and P. H. Fishman, *Biochim. Biophys. Acta*, 1974, **355**, 121.
[4] D. Robinson, *Enzyme*, 1974, **18**, 114.
[5] B. W. Nichols, in 'Plant Carbohydrate Biochemistry', Phytochemical Society Symposia, Series No. 10, ed. J. B. Pridham, Academic Press, New York, 1974, p. 97.
[6] R. J. Teichman, G. H. Takei, and J. M. Cummins, *J. Chromatog.*, 1974, **88**, 425.
[7] P. J. Evans and F. W. Hemming, *J. Chromatog.*, 1974, **97**, 293.
[8] M. Naoi, Y. C. Lee, and S. Roseman, *Analyt. Biochem.*, 1974, **58**, 571.
[9] R. Kannan, P. N. Seng, and H. Debuch, *J. Chromatog.*, 1974, **92**, 95.
[10] C. H. Chen and A. Nowotny, *J. Chromatog.*, 1974, **97**, 39.
[11] E. Heuser, K. Lipp, and H. Wiegandt, *Analyt. Biochem.*, 1974, **60**, 382.

amino-sugar and sialic acid.[12] In addition, information was obtained on the carbohydrate sequences, including chain branching. Phosphatidyldiglucosyldiglycerides reacted with 60% hydrogen fluoride to produce the diglyceride and the diglucosyldiglyceride.[13] No lyso-derivative could be detected, and the glycosidic linkage was stable to acid treatment. The separation of gangliosides from potential, water-soluble precursors has been achieved by enzymic digestion of nucleotide sugars, followed by a combination of chromatographic and electrophoretic techniques.[14] In order to clarify the function of gangliosides in excitable membranes, the interactions between gangliosides, proteins, and bivalent cations have been studied in both a two-phase and a single solvent system.[15] A tightly bound complex was formed containing about ten times as much calcium chloride as that calculated on a stoicheiometric basis. After ozonolysis of the olefinic bond of the sphingosine moiety of either globoside [β-D-Gal*N*Ac-(1 → 3)-α-D-Gal-(1 → 4)-β-D-Gal-(1 → 4)-β-D-Glc-ceramide] or the methyl ester of hematoside [α-*N*-glycolylneuraminyl-(2 → 3)-β-D-Gal-(1 → 4)-β-D-Glc-ceramide], the product was coupled to either aminoethylagarose or glass beads.[16] The resulting materials were used for the purification of anti-glycosphingolipid antibodies. Examination of the immunological properties of the antibodies showed that anti-globoside is directed towards the terminal 2-acetamido-2-deoxy-β-D-galactosyl-(1 → 3)-α-D-galactopyranosyl structure, whereas anti-hematoside is directed predominantly towards the sialyl residue of hematoside. Four D-galactosyl-lipids have been compared for immunological activity in model liposomal membranes.[17] The relative efficiencies, in terms of antigenic expression and immunogenicity, were in the order ganglioside > digalactosyldiglyceride > galactosylcerebroside > monogalactosyldiglyceride.

Animal Glycolipids and Gangliosides

The metabolism of glycolipids in the brains of patients with mucolipidosis II cell disease has been reported to be normal, and no specific accumulation could be attributed to an impaired activity of β-galactosidase.[18] Pathological brain cortex of a patient with visceral lipidosis contained twice the normal level of *N*-acetylneuraminyl-free glycolipids, due to the occurrence of glucocerebrosides, ceramide di-, tri-, and tetra-saccharides, and of high levels of Tay–Sachs ganglioside G_{M_2} and hematoside G_{M_3}.[19] Gangliosides and allied neutral glycosylceramides have been isolated from human-infant cerebral cortex and white matter, with $G_{D_{1a}}$ and G_{M_1} accounting for 70% of the total glycolipids in cerebral cortex.[20] In a search for new degradative pathways for D-glucosyl- and D-galactosyl-ceramides to explain the lack of accumulation of these glycolipids in the brain tissues of children with Krabbe's or Gaucher's diseases, it has been suggested that the

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[13] N. Shaw and A. Stead, *Biochem. J.*, 1974, **143**, 461.
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aetiology of these diseases involves a malfunction in one of the roles of the corresponding glycosidases.[21] The glycolipid-rich deposits that occur in individuals with Gaucher's disease have been found to contain significant amounts of β-glucosidase activity, which may be capable of glucocerebroside mobilization.[22] None of the gangliosides in neuronal membranes has been found to be susceptible to attack by exogenous neuraminidase.[23] Chromatograms of the gangliosides isolated from plaque tissue of multiple sclerotic brain material showed a decrease in a number of glycosylceramides compared to normal white matter.[24] Ceramide, sphingomyelin, mono-, di-, and tri-glycosylceramides, and ganglioside have been identified in human renal carcinoma.[25] Lactosyl- and D-galactosyl-sulphatides, components that are absent in normal human liver, have been isolated from patients with metachromatic leukodystrophy.[26] On the basis of methanolysis and methylation studies, both sulphatides were shown to be sulphated at O-3 of the D-galactosyl moiety. One of a number of gangliosides isolated from human spleen has been identified as a monosialolactoisohexaosylceramide (1).[27]

α-Neu*N*Ac-(2 → 3)-β-D-Gal-(1 → 4)-β-D-Glc*N*Ac-(1 → 3)-β-D-Gal-(1 → 4)-β-D-Glc*N*Ac-(1 → 3)-β-D-Gal-(1 → 4)-D-Glc-(1 → 1)-ceramide

(1)

A new glucosylsphingosine isolated from a sample of Gaucher's spleen has been identified as a D-glucosylceramide that is *N*-acylated with palmitic acid.[28] A microchemical assay for ganglioside G_{M_1} β-D-galactosidase has been developed using as substrate G_{M_1} containing a terminal D-[^{3}H]galactosyl residue.[29] In a study of four human-tumour cell lines, a significant reduction was observed in the incorporation of L-[^{3}H]fucose into the fucolipids, probably reflecting changes in the glycosyltransferase activities.[30] Data have been produced to show that alterations in the metabolism of fucosylglycolipids in cells transformed by a temperature-sensitive mutant of murine sarcoma virus are related directly to the expression of the transformed state and not simply as a result of infection.[31]

A much higher concentration of glycolipids has been detected in the urinary-tract transitional epithelium of mammalian kidney than in the connecting tubules.[32] A ceramide tetrasaccharide (2) from human-erythrocyte membranes gave a cross-reaction with antiserum to type XIV pneumococcal polysaccharide.[33] Two gangliosides with identical carbohydrate skeletons, one having *N*-acetyl-

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neuraminic acid, and the other having D-galactose, substituted at O-3 of their terminal D-galactosyl residue, were also characterized.[34]

$$\beta\text{-D-Gal-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}N\text{Ac-}(1 \rightarrow 3)\text{-}\beta\text{-D-Gal-}(1 \rightarrow 4)\text{-D-Glc-}(1 \rightarrow 1)\text{-ceramide} \quad (2)$$

Three human-erythrocyte blood-group antigens, P, P_1, and p^K, have been identified as glycosphingolipids.[35] The p^K antigen was identified as a ceramide trihexoside, α-D-Gal-(1 → 4)-β-D-Gal-(1 → 4)-D-Glc-ceramide, and the P antigen was identified as the corresponding tetrasaccharide bearing a (1 → 3)-linked 2-acetamido-2-deoxy-β-D-galactosyl residue attached to the non-reducing, terminal D-galactosyl residue. Contrary to a previous proposal, it was suggested that the p^K antigen is the biosynthetic precursor of the P antigen, but that neither is a precursor of the P_1 antigen. Glycosphingolipids isolated from the total lipids of human female and male thyroid glands exhibited marked differences, suggesting that their biosynthesis is under hormonal control.[36] The similarities of the glycosphingolipids present in human thyroid and kidney were discussed in relation to a possible role of the glycolipids in the transport of ions, which is a common function of these organs.

myo-Inositol present in animal tissues has been suggested to be either an inhibitor or a regulator of the *in vivo* hydrolysis of ceramide lactoside and ceramide trihexoside by the corresponding galactosidases.[37] The Forssman glycolipid hapten isolated from horse kidneys has been analysed by m.s. of undegraded lipid derivatives.[38] Even without the customary degradative studies, it was possible to confirm the original proposal[39] that the glycolipid is a pentaglycosylceramide possessing the sequence hexosamine–hexosamine–hexose–hexose–hexose–ceramide. A methylated, bovine-brain disialoganglioside has been subjected to m.s. analysis, which revealed intense ions for the two methylated sialic acid residues and also ions corresponding to several or all of the six sugars and to the ceramide moiety.[40] Several series of primary and rearrangement ions provided information on the sequence of units and on branching of the carbohydrate chain. Six chromatographically distinct gangliosides have been isolated from bovine mammary glands and fat-globule membranes, and the carbohydrate sequences of three of them were determined.[41] A study of the distribution of molecular species of monoglycosylceramides in different parts of bovine digestive tract has indicated that all the regions studied contain D-galactosyl- and D-glucosylceramides.[42] Major differences have been reported in the sphingosine and fatty-acid patterns of the major gangliosides of bovine retina.[43] Four major gangliosides, G_{M_1}, $G_{D_{1d}}$, $G_{D_{1b}}$, and G_{T_1}, have been detected in bovine optic nerve, and both the

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fatty-acid and sphingosine patterns of these gangliosides differ from those of retinal gangliosides.[44] Further chemical and immunochemical investigations on the Forssman globoside obtained from caprine erythrocyte stroma have supported the original structural findings.[45] Desulphation of the mono-, di-, and tri-hexose sulphatides of hog gastric mucosa has yielded products identified as D-galactosyl-ceramide, lactosylceramide, and D-galactosyl-lactosylceramide, respectively.[46] In each case, the sulphate group was found to be linked to the non-reducing D-galactosyl residue through O-3. An L-fucose-containing ceramide tetrasaccharide, purified from hog stomach mucosa by a combination of ion-exchange chromatography and t.l.c., was found to exhibit blood-group H activity.[47] The structure (3) of this glycolipid was proposed on the basis of partial acidic hydrolysis, sequential enzymic degradations, and methylation studies.

α-L-Fuc-(1 → 2)-α-D-Gal-(1 → 3)-β-D-Gal-(1 → 4)-D-Glc-(1 → 1)-ceramide

(3)

Similar techniques have been applied to the identification of the L (4) and U (5) blood-group A glycolipids of hog gastric mucosa.[48]

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

(4)

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

(5)

Glycosphingolipids of lymphoid tissues, bone-marrow cells, mixed blood leucocytes, and separated lymphocytes and granulocytes from pigs showed marked differences in composition between blood polymorphonuclear neutrophils and bone-marrow cells.[49] The purification and composition of a digalactoside ceramide from porcine pancreas has been reported.[50] The interaction of protamine with gangliosides has been used successfully to remove endogenous gangliosides from preparations of sheep-brain neuraminidase.[51] Double-labelling of the Forssman hapten from sheep erythrocytes with ^{3}H and ^{14}C, followed by enzymic

[44] M. Holm and J. E. Månsson, *F.E.B.S. Letters*, 1974, **45**, 159.
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hydrolysis, has been used to determine the anomeric configurations of the linkages in the glycosphingolipid.[52] Identical results were obtained using chemical methods, and it was concluded that the carbohydrate sequences are the same as for those of the corresponding haptens from horse and canine kidneys. The neutral glycosphingolipids and gangliosides of cell-membrane particulate fractions from normal and allograft-rejected canine kidneys have been examined.[53] A number of glycolipids were shown to be present in increased concentrations, and others in decreased concentrations, in the rejected kidney; the structure of each glycolipid was investigated by classical methods. The glycosphingolipids of the kidney membrane were shown by complement fixation to have little or no antibody-binding capacities when tested against sera obtained from transplant recipients after removal of the rejected-kidney allograft.

The isolation of ganglioside–protein complexes and free gangliosides from rat brain has been shown to depend on the nature of the starting material, since whole-brain tissues yielded only ganglioside–protein complexes, whereas mitochondrial, microsomal, and synaptosome-enriched fractions yielded both free and protein-bound gangliosides.[54] Most of the constituent gangliosides of membranes of the microsomal fraction of rat brain were found to be susceptible to the action of a neuraminidase from *Clostridium perfringens*, although a small proportion of the gangliosides were not exposed to the action of this enzyme.[55] Moreover, the endogenous sialyltransferases were unable to reincorporate sialic acid groups into partially desialylated gangliosides that remained attached to the membranes. These findings indicated that recently synthesized polysialogangliosides (completed *in vitro*) are located in the membranes in positions less accessible to neuraminidase than are those synthesized earlier and which are present at the outset of the experiment. The *in vitro* incorporation of D-[^{14}C]glucose into the tetrasialoganglioside G_{Q_1} of rat brain has been shown to require a specific and a separate enzyme system from the proposed precursor gangliosides G_{M_1}, $G_{D_{1a}}$, $G_{D_{1b}}$, and $G_{T_{1b}}$.[56] The enzymic sulphation of a number of D-galactose-containing sphingolipids in developing rat brain has been shown to involve the transfer of sulphate from adenosine 3′-phosphate 5′-phosphosulphate to O-3 of D-galactosyl residues.[57] The biosynthesis and concentrations of D-galactosyldiglycerides in oligodendroglial, astroglial, neuronal, and myelin-enriched fractions of rat brain have been studied.[58] It was suggested that oligodendroglial cells are the site of synthesis of myelin, a constituent of the central nervous system, and that there is a temporal relation between the sites of synthesis and deposition of myelin. As a result of *in vivo* studies on the biosynthesis of rat-brain ceramide and cerebroside, it has been suggested that the hydroxy-fatty-acid ceramide may not be the physiological precursor of the hydroxy-fatty-acid D-galactosylceramide.[59] When rats were rendered hypothyroid by treatment with methimazole, both *in vivo* and *in*

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vitro incorporation of D-[1-^{14}C]galactose into brain cerebrosides was significantly lowered.[60] A glycero-D-galactolipid, which is synthesized *in vitro*, was found at reduced levels in hypothyroid rats.

A general review has been published on the biosynthesis of brain gangliosides.[61] A D-galactosyltransferase isolated from rat kidneys catalysed the synthesis of di-D-galactosylceramide from UDP-D-galactose and D-galactosylceramide; this enzyme was found to differ from that catalysing the addition of D-galactose to lactosylceramide to form D-galactosyl-D-galactosyl-D-glucosylceramide.[62] The classes of glycosphingolipids identified in unfractionated rat liver and in lysosomes included a hematoside containing *N*-acetylneuraminic acid and tetrahexosylceramides containing 2-amino-2-deoxy-D-galactose.[63] It was demonstrated that some classes of glycosphingolipid are concentrated in the secondary lysosomes to a degree expected of markers for primary lysosomes and plasma membrane. Fractions of the Golgi apparatus from rat liver have been found to contain all the glycosyltransferases which catalyse the *in vitro* synthesis of gangliosides.[64] Plasma-membrane fractions displayed negligible glycolipid: glycosyltransferase activities. As with membrane glycolipids, gangliosides appear to be glycosylated in the endoplasmic reticulum and Golgi apparatus during transport to the surface membrane. The trihexosylceramides synthesized from lactosylceramide and rat-kidney UDP-D-galactose D-galactosyltransferase have been shown to consist of two positional isomers in which the D-galactopyranosyl units are (1 → 3)- and (1 → 4)-linked.[65] A major glycolipid, 2-*O*-acyl-1-*O*-alkyl-3-*O*-(β-D-galactopyranosyl 3-sulphate)-glycerol, isolated from rat testes, although not hydrolysed by human arylsulphatase B, was found to be a physiological substrate for arylsulphatase A.[66] The occurrence of UDP-D-Gal*N*Ac: globoside α-D-Gal*N*Ac transferase activity in guinea-pig microsomal preparations has been reported.[67] The product of the enzymic reaction, a Forssman hapten, was identified by enzymic hydrolysis and immunoprecipitin reactions after purification. The enzyme gave a maximum incorporation of the amino-sugar from UDP-2-acetamido-2-deoxy-D-galactose with human-kidney trihexosylceramide and appreciable incorporations with human-kidney globoside and equine-erythrocyte hematoside.[68] When glycosphingolipids that had been linked covalently to glass particles were placed in contact with baby hamster-kidney fibroblasts or hamster embryo cells, glycosylation of the molecules took place.[69] Synthesis occurred under physiological conditions, with or without exogenous nucleotide diphosphate D-glucose, and was ascribed to the activation of glycosyltransferases localized at the surface of the cells. The ganglioside composition of a non-

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producer subclone in mouse cells transformed by murine sarcoma virus was found to be altered significantly in comparison to the non-transformed parent clone, due to the complete absence of a specific D-galactosyltransferase required for the synthesis of mono- and di-sialogangliosides.[70] A study has been made of the different kinds of glycosyltransferases on the cell surfaces of normal and transformed mouse fibroblasts.[71] Kinetic parameters and optimal ion concentrations of the glycosyltransferases were determined when whole cells were incubated with nucleotide sugars. Mono-D-glucosyl- and mono-D-galactosyl-ceramides have been identified as the major glycolipids of bullfrog oxyntic-cell microsomes by quantitative t.l.c.[72] D-Glucosyl- and D-galactosyl-ceramides have been synthesized by incubation of an embryonic, chicken-brain microsomal fraction with stearoyl-coenzyme A and the corresponding D-glucosyl- and D-galactosyl-sphingosines; the formation of free sphingosine, by enzymolysis of the glycosylsphingosines prior to the synthesis of the cerebrosides, was not required.[73] The properties have been described of a glycosyltransferase from embryonic chicken brain that is involved in the synthesis of D-glucosylceramide from ceramide and UDP-D-glucose.[74] Hen-oviduct membranes were shown to catalyse the transfer of D-[^{14}C]xylose from UDP-D-[^{14}C]xylose into a xylosyl-lipid, which was identified as a xylosylphosphoryl polyisoprenol by chemical studies.[75] Egg-grown influenza virus has been shown to contain a Forssman hapten.[76] Tissues of the digestive gland of the starfish *Distolasterias nipon* contain a sialoglycolipid, whose structure (6) was elucidated using classical chemical methods.[77]

α-Neu*N*Ac-(2 → 8)-α-Neu*N*Ac-(2 → 8)-α-Neu*N*Ac-(2 → 3)-
D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

(6)

Plant and Algal Glycolipids

Acylated D-galactosides of ethylene glycol have been described as a new type of natural lipid in ripening maize seed.[78] An acyl derivative of 1-*O*-β-D-galactopyranosyl ethylene glycol was isolated from maize seed, and derivatives thereof were characterized by g.l.c.–m.s. Monogalactosyl-, digalactosyl-, trigalactosyl-, and quinovosyl-diglycerides have been isolated from pumpkin.[79] The structure of the trigalactosyldiglyceride was assumed to be 1,2-diacyl-3-*O*-[α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl]-*sn*-glycerol. An

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acylated digalactosyldiglyceride has been isolated from spinach-leaf homogenates; chromatographic and enzymic studies identified it as a 1,2-diacyl-3-*O*-[6′-*O*-(6″-*O*-acyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-*sn*-glycerol.[80] The ceramide di- and tri-hexosides of wheat flour showed properties compatible with the structures β-D-mannopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 1)-ceramide and β-D-mannopyranosyl-(1 → 4)-β-D-mannopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 1)-ceramide, respectively.[81] The intracellular distributions and changes of activity during the greening of etiolated *Vicia faba* seedlings have been investigated for a number of enzymes contributing to the biosynthesis of galactolipid precursors.[82] A number of glycolipids which are specific for heterocyst-forming algae have been isolated from the heterocyst envelope of *Anabaena cylindrica*.[83] Two non-saponifiable lipids were identified as 1-*O*-α-D-glucopyranosyl-3,25-hexacosanediol and 1-*O*-α-D-glucopyranosyl-3,25,27-octacosanetriol by m.s.

Microbial Glycolipids

A heptose-containing pentaglycosyldiglyceride has been identified among the lipids of *Acholeplasma modicum*; a structure tentatively proposed was D-Gal-D-Gal-D-*glycero*-D-*manno*-heptosyl-D-Glc-D-Glc-diglyceride, with the diglyceride-terminating moiety possessing the structure α-D-Glc*p*-(1 → 2)-D-Glc*p*-*sn*-1,2-diglyceride.[84] A 1-*O*-D-galactosyldiglyceride has been identified among the glycolipids present in *Actinomyces viscosus*; analysis of the fatty acids showed them to consist largely of palmitic and stearic acids and their mono-unsaturated homologues.[85] Growth of some species of *Arthobacter*, *Corynebacteria*, *Nocardia*, and *Mycobacteria* on D-fructose produced two novel glycolipids, identified as D-fructose 6-corynomycolate and D-fructose 1,6-dicorynomycolate.[86] Phosphogalactolipids have been isolated from cultures of *Bifidobacterium bifidum* after inhibition of cell-wall synthesis.[87] From chemical analyses and enzymic degradations, one of the components was identified as 3-*O*-[6-(*sn*-glycerol 1-phosphate)-β-D-galactofuranosyl]-*sn*-1,2-diglyceride. A particulate fraction isolated from *B. bifidum* synthesized five ^{14}C-labelled D-galactosyldiglycerides from UDP-D-[^{14}C]galactose and 1,2-dipalmitin.[88] Mono- and di-D-galactosyldiglycerides appeared to be precursors of the di- and tri-D-galactosyldiglycerides, respectively. A purified, cell-wall glycolipid from *Flavobacterium thermophilum*, comprising D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, glycerol, and fatty-acid esters and amides (2 : 1 : 1 : 1 : 2 : 1), has been examined, and the structure (7) was proposed on the basis of chemical and enzymic investigations.[89]

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D-Gal*f*-(1 → 2)-D-Gal*p*-(1 → 6)-D-Glc*N*(15-methylhexadecanoyl)-
(1 → 2)-D-Glc*p*-diglyceride

(7)

A particulate enzyme preparation from *Mycobacterium smegmatis* catalysed the incorporation of D-mannose from GDP-D-[^{14}C]mannose and of D-glucose from UDP-D-[^{14}C]glucose into endogenous lipid-acceptors, with the production of glycosylphosphoryl polyprenols.[90] Evidence was also obtained for the formation of a monoglycosyldiglyceride and a diglycosyldiglyceride.[91] The preparation of an adjuvant-active, tuberculin-free peptidoglycolipid from human tubercle bacilli has been reported.[92] A preliminary report has indicated the existence of a close serological relationship between the glycolipids of *Mycoplasma pneumoniae* and those of spinach chloroplasts.[93] The glycolipids isolated from *Salmonella minnesota* and *S. typhimurium* have been shown to possess similar compositions; passive haemagglutination tests demonstrated a cross-reactivity, and it was concluded that the glycolipids probably have identical immunodeterminant groups.[94] An enzyme from a membrane fraction of *Shigella flexneri* was able to catalyse the transfer of hexose from UDP-D-glucose and UDP-D-galactose to polyprenol monophosphate, but no transfer took place when UDP-D-glucuronic acid and UDP-2-acetamido-2-deoxy-D-glucose were used as substrates; ficaprenol (mainly C_{55}) phosphate was the most effective lipid acceptor of D-glucose.[95] D-Glucuronosyldiglycerides have been isolated from the total cellular lipids of a *Streptomyces* species that produces the antibiotic aureolic acid.[96]

The present evidence for gangliosides as membrane receptors for tetanus toxin, cholera toxin, and serotonin has been reviewed.[97] The biochemical, biological, and immunological properties have been described for complexes formed by choleragen (from *Vibrio cholerae*) and the ganglioside $G_{G_{tet}}1\{\beta$-D-Gal-(1 → 3)-β-D-Gal*N*Ac-(1 → 4)-[α-Neu*N*Ac-(2 → 3)]-β-D-Gal-(1 → 4)-D-Glc-ceramide$\}$.[98] The effects on this interaction of variations in the sialo-oligosaccharide and in the lipid moiety of the ganglioside were investigated.

A glycolipid antigen isolated from aggregation-competent cells of *Dictyostelium discoideum* comprises 2-acetamido-2-deoxy-D-glucose, L-fucose, and D-mannose (12 : 5 : 2), 4-hydroxysphinganine, and behenic acid.[99] Cells of a non-aggregating mutant contained a glycolipid exhibiting different biological and chemical properties, notably in having a much lower content of L-fucose and in having part of the amino-sugar in a non-reducing, terminal position. The major phosphosphingolipid isolated from *Neurospora crassa* has been characterized as a di-

[90] J. C. Schultz and A. D. Elbein, *Arch. Biochem. Biophys.*, 1974, **160**, 311.
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[99] O. H. Wilhelms, O. Lüderitz, O. Westphal, and G. Gerisch, *European J. Biochem.*, 1974, **48**, 89.

(inositolphosphoryl)ceramide, and a minor component was tentatively identified as a ceramide tetrahexoside, *viz.* (D-Gal)$_3$-D-Glc-*N*-hydroxytetracosonylhydroxysphinganine.[100] Sphingolipids have been identified as the dominant glycolipids of baker's yeast, which also contains relatively large quantities of ceramides, including a di(inositolphosphoryl)mannosylceramide.[101] A microsomal fraction from *Schizosaccharomyces pombe* catalysed the transfer of D-mannosyl residues to endogenous acceptors, with the formation of four types of lipid.[102] Alkaline degradation provided evidence for the presence of a D-mannosyldiglyceride and a family of lipids carrying oligosaccharide chains of four or more units.

[100] R. L. Lester, S. W. Smith, G. B. Wells, D. C. Rees, and W. W. Angus, *J. Biol. Chem.*, 1974, **249**, 3388.
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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

Polysaccharides.—A fraction from a synthetic, linear dextran (D-8, number average mol. wt. 2.69×10^4) and a B 512 dextran fraction (D-40) having a similar molecular-weight distribution, but also possessing branch-points (1 per 20 residues) and short side-chains, have been compared as antigens.[1] In Ouchterlony gel double diffusion, the two dextrans gave single and identical precipitin lines, and in single radial immunodiffusion comparable amounts of anti-dextrans gave precipitin haloes of similar size. It was concluded that D-8 is a homodeterminant antigen with many determinants and that the antigenic determinants of both the synthetic and B 512 dextrans are repeated along the molecular chain.

Both poly- and oligo-fructans were synthesized when conidia of *Aspergillus sydowi* were incubated with sucrose.[2] The polyfructan was shown to have a mol. wt. of approximately 2.0×10^7 (*i.e.* comparable to levans), with the chains comprising (2 → 1′)-linked β-D-fructofuranosyl residues as in inulin of higher plants. The polyfructan differs in structure from known polyfructans.

Fusion of 1,2,3,4-tetra-*O*-acetyl-α-D-mannopyranose with a catalytic amount of toluene-*p*-sulphonic acid furnished 6-*O*- and 4-*O*-α-D-mannopyranosyl-D-mannose after deacetylation, but no β-linked disaccharides were detected.[3] When the corresponding β-tetra-acetate was fused with zinc chloride as catalyst, higher oligomers were formed as well as a D-mannan (DP 10) comprising mainly α-(1 → 6)-linkages. With 5% of zinc chloride as catalyst, the α-tetra-acetate formed oligosaccharides, but yielded a smaller proportion of a (1 → 6)-linked D-mannan.

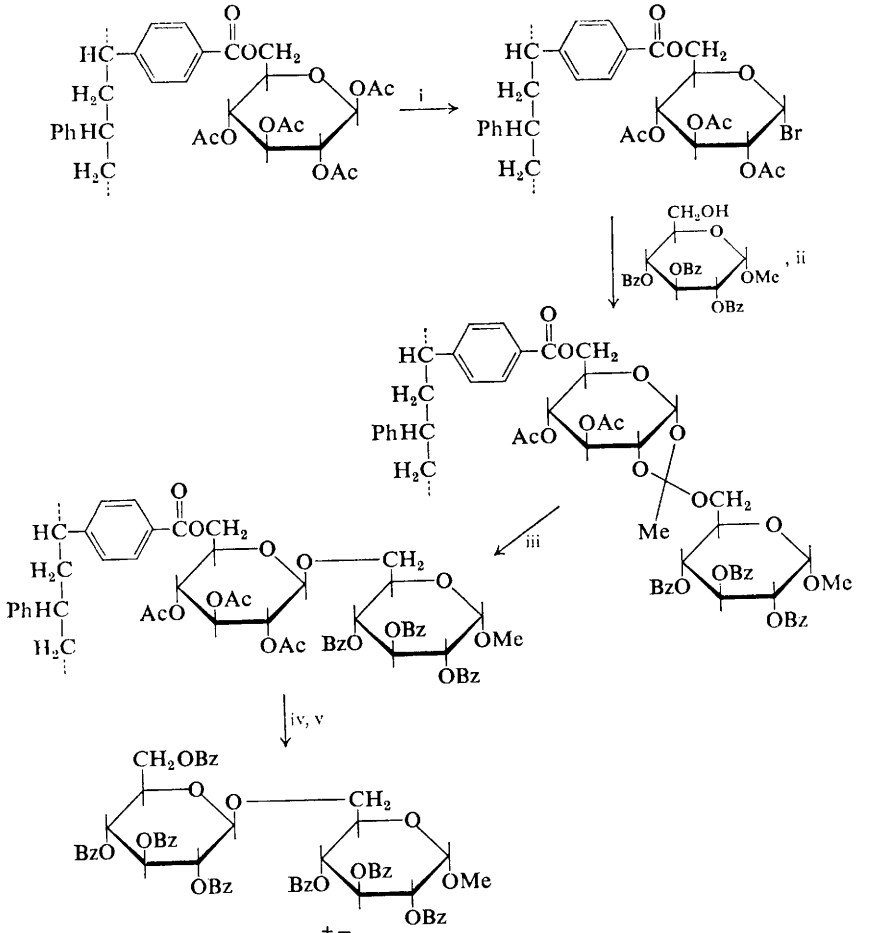
Pullulan can be synthesized from sucrose by acetone-dried cells of *Pullularia pullulans* and from UDP-D-glucose by a cell-free system prepared from the organism.[4] ATP was essential for the synthesis of pullulan, although ADP-D-glucose could not replace UDP-D-glucose. The formation of pullulan was determined by precipitation of the polysaccharide with ethanol and the measurement of maltotriose liberated therefrom by the pullulanase from *Aerobacter*

[1] W. Richter, *Internat. Arch. Allergy*, 1974, **46**, 438.
[2] G. Kawai, H. Taniguchi, and M. Nakamura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2111.
[3] E. O'Brien, E. E. Lee, P. S. O'Colla, and U. Egan, *Carbohydrate Res.*, 1974, **32**, 31.
[4] R. Taguchi, Y. Sakano, Y. Kikuchi, M. Sakuma, and T. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1635.

aerogenes. A lipid containing D-glucosyl residues was formed during synthesis of the polysaccharide, and the involvement of a lipid intermediate in the synthesis of pullulan was examined.

Oligosaccharides.—Oligofructans produced concomitantly with polyfructan on incubation of the conidia of *Aspergillus sydowi* with D-fructose have been shown to belong to the 1-kestose series having the general formula (1^F-(D-fructosyl)$_n$-sucrose).[2]

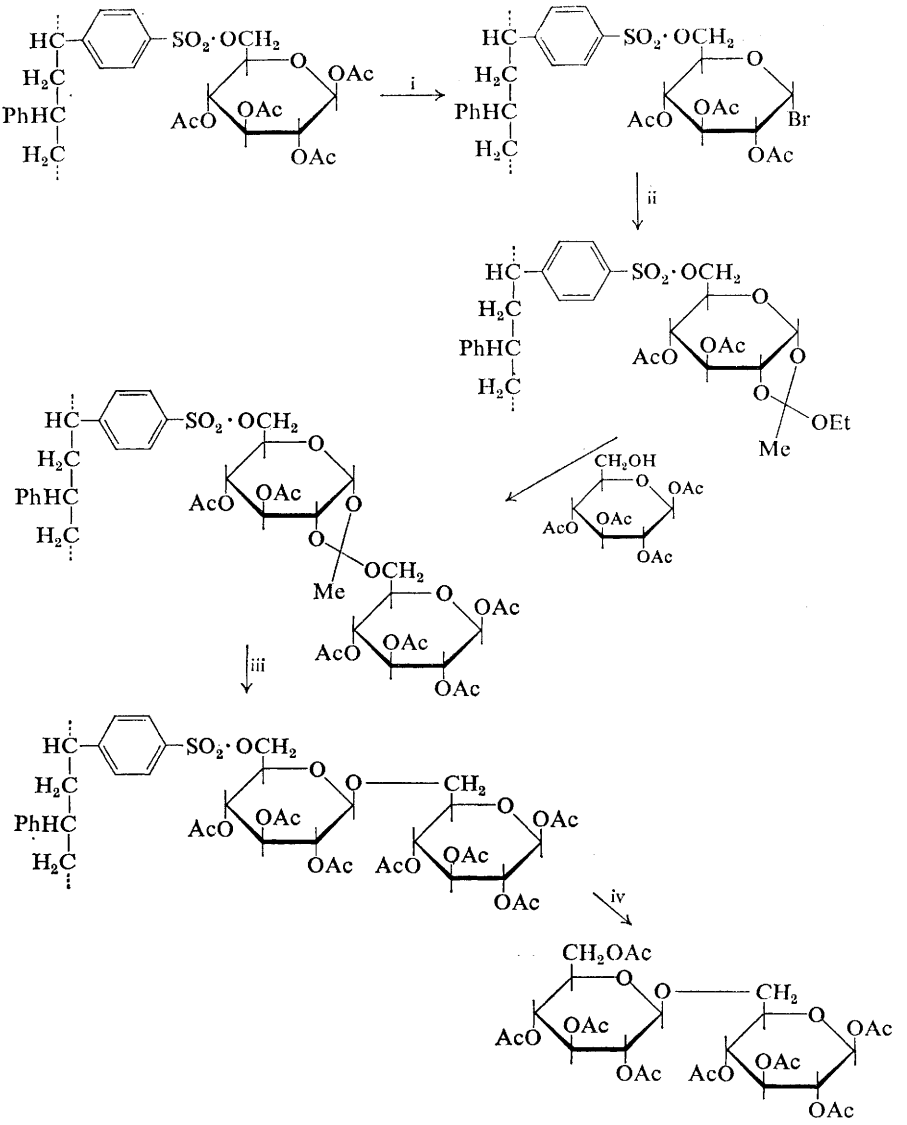
Styrene-based copolymers incorporating either 1,2,3,4-tetra-*O*-acetyl-6-*O*-(4-vinylbenzoyl)-β-D-glucopyranose or the 6-*O*-(4-vinylphenylsulphonyl) analogue have been prepared and used as soluble supports in the synthesis of β-gentiobiose derivatives, as outlined in Schemes 1 and 2, respectively.[5]



Reagents: i, HBr–AcOH; ii, $Bu_4\overset{+}{N}\overset{-}{Br}$–*sym*-collidine; iii, lutidinium perchlorate–TsOH; iv, NaOMe; v, BzCl–py

Scheme 1

[5] R. D. Guthrie, A. D. Jenkins, and G. A. F. Roberts, *J.C.S. Perkin I*, 1973, 2414.

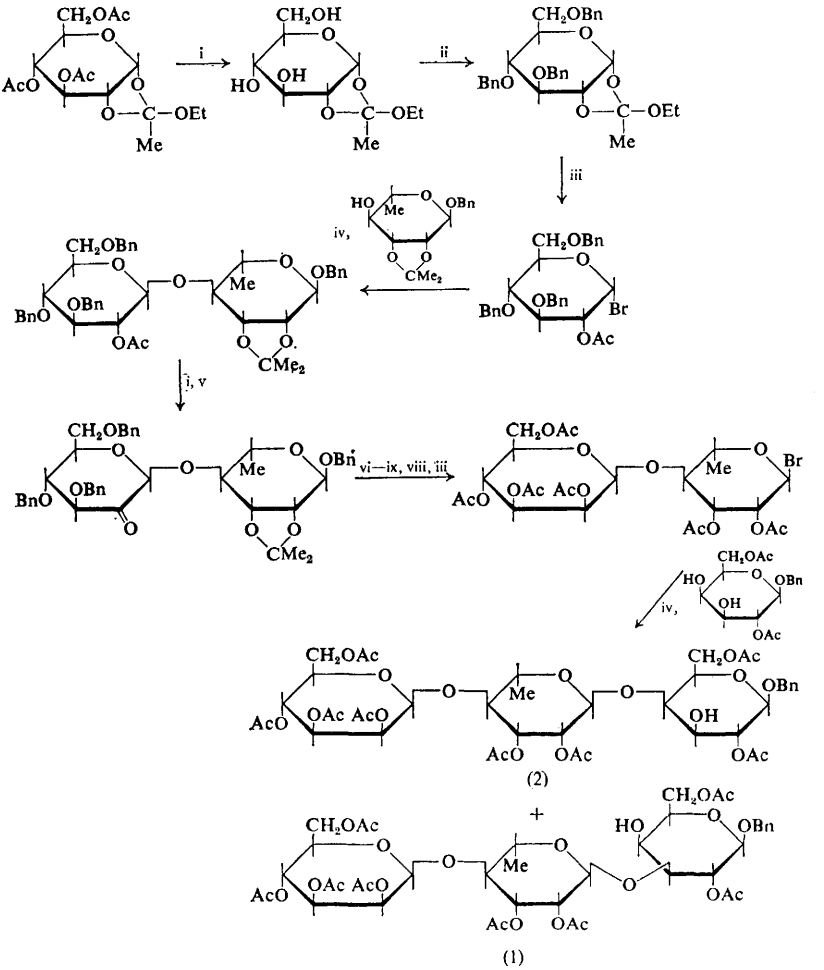


Reagents: i, HBr–AcOH; ii, $Bu_4\overset{+}{N}\overset{-}{Br}$–*sym*-collidine–EtOH; iii, pyridinium perchlorate; iv, KOAc–DMF

Scheme 2

The synthesis has been described of derivatives of the trisaccharide repeating unit, *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-α-D-galactopyranose, of the *O*-specific polysaccharide from *Salmonella anatum*, which appears to be a common component for all *Salmonella* serotypes belonging to

group E.[6] The synthesis involved the construction of the trisaccharide (1), requiring the formation of a β-D-mannopyranosyl linkage and the introduction of a 3-substituted D-galactosyl residue, as shown in Scheme 3. A trisaccharide (2) containing two (1 → 4)-linkages was also isolated from the final condensation.



Reagents: i, MeONa–MeOH; ii, BnCl–AcOH; iii, HBr–AcOH; iv, $Hg(CN)_2$; v, DCC–DMSO–H^+; vi, H_2–Pt; vii, H_2–Pd; viii, Ac_2O–py; ix, CF_3CO_2H

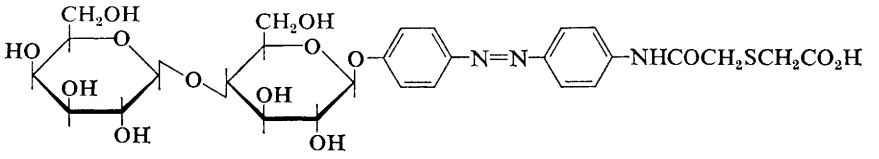
Scheme 3

Glycoproteins.—A hapten (3) containing 4-azophenyl β-lactoside in the terminal portion and a tritiated form thereof have been synthesized.[7] The hapten (3) was coupled to poly(L-lysine) and to bovine immunoglobulin by carbodi-imide-assisted

[6] N. K. Kochetkov, B. A. Dmitriev, O. S. Chizov, E. M. Klimov, N. N. Malysheva, A. Y. Chernyak, N. E. Bayramova, and V. I. Torgov, *Carbohydrate Res.*, 1974, **33**, C5.

[7] P. V. Gopalakrishnan, W. S. Huges, Y. Kim, and F. Karush, *Immunochemistry*, 1973, **10**, 191.

reactions, and immunization experiments with the pseudoglycoprotein produced immunoglobulin IgG antibodies of functional homogeneity. Whereas the poly(L-lysine)-based material proved to be unsuitable as an immunogen for anti-hapten activity, the immunoglobulin-based material was an effective antigen. A related pseudoglycoprotein was obtained by coupling (3) to bovine serum albumin.



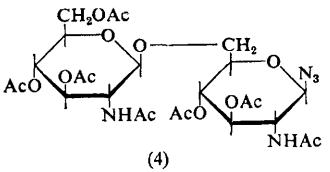
(3)

Glycopeptides.—Novel analogues of 2-acetamido-1-*N*-(L-aspart-4-yl)-2-deoxy-β-D-glucopyranosylamine, the glycopeptide junction of the immunoglobulins, have been synthesized as potential regulators of the synthesis, secretion, and function of the immunoglobulins.[8] The 2-amino- and carboxy-groups of the L-aspartyl residue were incorporated into hydantoin, thiohydantoin, and dioxopiperazine systems, and the products were converted into N^2-toluenesulphonamides to simulate the neighbouring peptide linkages of this junction. The amide linkage in 2-acetamido-1-*N*-(L-aspart-4-yl)-2-deoxy-β-D-glucopyranosylamine was thereby replaced by glycosidic and by sulphonamidic linkages, the latter compounds representing a new type of glycosylamine. The chemical stability of these linkages was examined.

2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (4), obtained by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl bromide with either 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide or the corresponding 6-*O*-trityl derivative, was reduced in the presence of Adams' catalyst to the disaccharide amine.[9] Condensation of (4) with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate afforded 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-acetyl-1-*N*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-yl]-2-deoxy-β-D-glucopyranosylamine (5), which on hydrogenation and saponification gave (6). The bis(glycosylamine) (7) was obtained as a by-product on reduction of (4).

The glycosyl tetra- and penta-peptides (8) and (9) and related derivatives have been synthesized by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonyl-L-aspart-4-yl)-2-deoxy-β-D-glucopyranosylamine with appropriately protected tetra- and penta-peptides and amino-acids.[10] The amino-acid sequences of (8) and (9) correspond to the sequences 34—37 and 34—38 adjacent to the glycopeptide linkage of ribonuclease.

[8] T. Y. Shen, J. P. Li, C. P. Dorn, D. Ebel, R. Bugianesi, and R. Fecher, *Carbohydrate Res.*, 1972, **23**, 87.
[9] E. Walker and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **32**, 145.
[10] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1974, **32**, 37.



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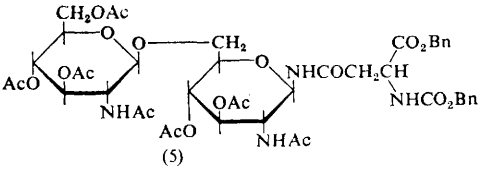

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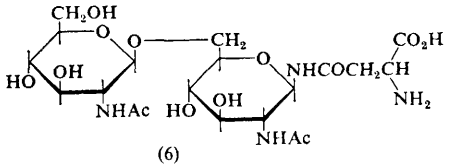

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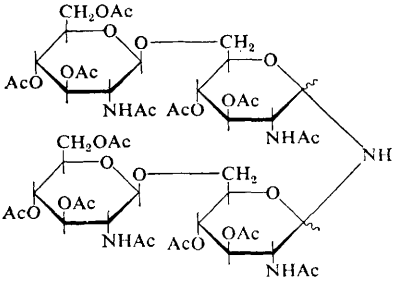

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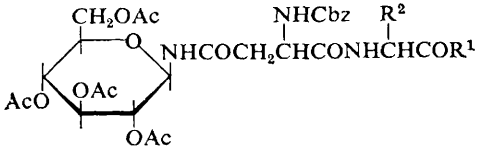


(8) R^1 = NHCHCONHCHCO$_2$CH$_2$C$_6$H$_4$·NO$_2$-*p*; R^2 = CH_2CHMe_2

CHOH (CH$_2$)$_4$

Me NHTs

(9) R^1 = NHCHCONHCHCONHCHCO$_2$CH$_2$C$_6$H$_4$·NO$_2$-*p*; R^2 = CH_2CHMe_2

CHOH (CH$_2$)$_4$ CH$_2$

Me NHTs CO$_2$CH$_2$C$_6$H$_4$·NO$_2$-*p*

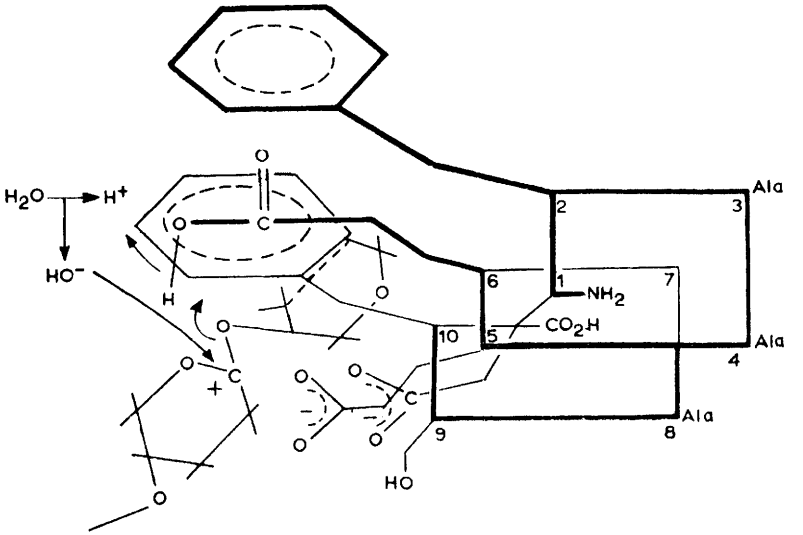
Enzymes.—Based on the structure of lysozyme, it was predicted that the decapeptide (10) would have a tendency to form an α-helix in a conformation having the

L-Glu-L-Phe-L-Ala-L-Ala-L-Glu-L-Glu-L-Ala-L-Ala-L-Ser-L-Phe

(10)

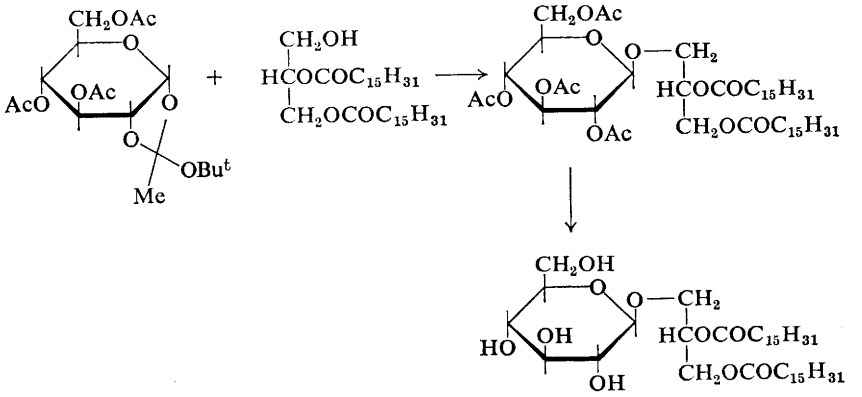
carboxy-group of L-glutamic acid-6 in a hydrophobic environment, since it would be flanked above and below by the aromatic rings of L-phenylalanine-2 and -10 and by the adjacent L-alanine-7 residue (Scheme 4).[11] L-Glutamic acid-5, on the other hand, would be expected to be in a hydrophilic environment such that its carboxy-group or that of L-glutamic acid-1 and the carboxy-group of L-glutamic acid-6 could provide a catalytic site in the enzyme model (see Scheme 4). The synthetic decapeptide hydrolysed chitin and dextran at approximately equal rates; although these enzymic activities were low, they were significant.

[11] P. K. Chakravarty, K. B. Mathur, and M. M. Dhar, *Experientia*, 1973, **29**, 786.



Scheme 4

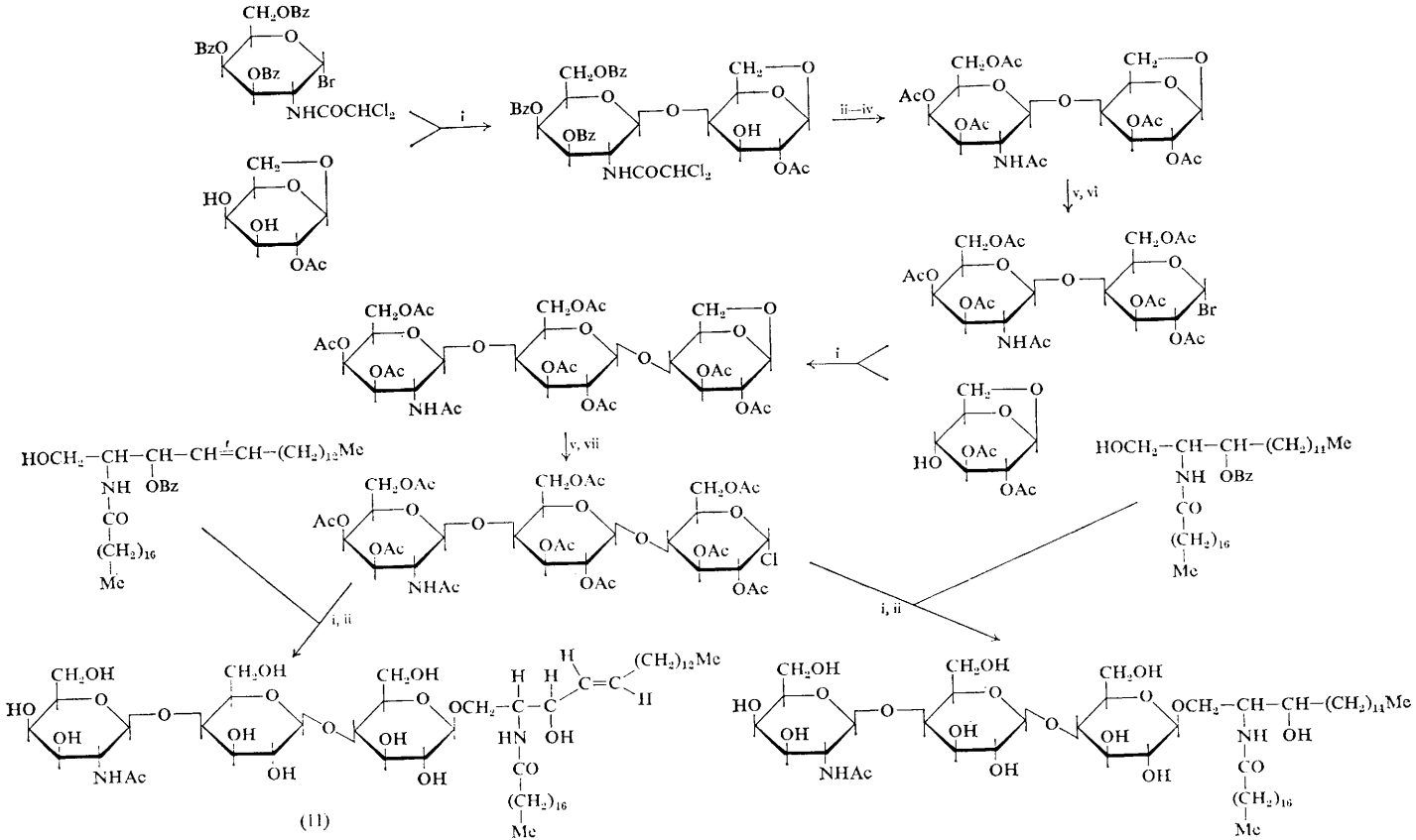
Glycolipids and Gangliosides.—Syntheses of mono- and di-glycosyldiglycerides from 1,2-di-*O*-acyl- and 1,2-*O*-isopropylidene-*sn*-glycerols and 2,5-*O*-methylene-D-mannitol by the orthoester method have been described (see Scheme 5).[12]



Scheme 5

D-Glucosyl- and D-galactosyl-ceramides have been synthesized by incubation of a fraction from the microsomes of chicken-embryo brains with stearoyl-coenzyme A and either D-glucosyl- or D-galactosyl-sphingosine.[13] The formation of free sphingosine, by enzymic cleavage of the glycosylsphingosines, was not a prerequisite for the synthesis of the cerebrosides.

[12] V. I. Shvets, A. I. Bashkatova, and R. P. Evstigneeva, *Chem. and Phys. Lipids*, 1973, **10**, 267.
[13] J. A. Curtino and R. Caputto, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 142.



Reagents: i, $Hg(CN)_2$; ii, $Ba(OMe)_2$; iii, $Ba(OH)_2$; iv, Ac_2O–py; v, AcOH–Ac_2O–H_2SO_4; vi, HBr–AcOH; vii, AcOH–Ac_2O–HCl

Scheme 6

Radiolabelled D-glucocerebroside was obtained when the β-D-glucosidase from calf spleen was incubated with 4-methylumbelliferyl β-D-glucopyranoside and [^{14}C]ceramide, but neither D-glucose nor methyl β-D-glucopyranoside were effective donors.[14] The labelled glycolipid also acted as a substrate for the β-D-glucosidase.

The total synthesis of the natural 'Tay–Sachs' disease globoside (11) has been achieved by the sequence of reactions shown in Scheme 6.[15] A glycolipid containing the racemic aglycone and a dihydro-derivative of (11) were also synthesized.

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

A review of chemically reactive derivatives of polysaccharides has covered short-chain 'primary' and dye derivatives, cross-linked polysaccharides, graft co-polymers of polysaccharides, water-insoluble enzymes, immunoadsorbents and affinity-chromatography matrices as polysaccharide derivatives, nucleic acid and antibiotic derivatives of polysaccharides, non-covalent complexes of polysaccharides, and derivatives of cycloamyloses.[16]

A review of affinity chromatography has described matrices based on polysaccharides, including those that are available commercially.[17] Another review, which concentrates on chemical aspects of affinity chromatography, has described the chemistry of polysaccharide-based affinity matrices and has also dealt with the applications of affinants to the purification of enzymes, carbohydrates, glycoproteins, nucleic acids, peptides, antigens, binding-, transport-, and receptor-proteins, intact cells, polysomes, and viruses.[18] A book covering various aspects of affinity chromatography also mentions matrices based on polysaccharides.[19]

The production of water-insoluble derivatives of enzymes by covalent attachment to polysaccharides has been described briefly,[20] whereas the production of polysaccharide derivatives for specialized applications, including the immobilization of enzymes, the production of immunoadsorbents, and use in radioimmunoassays, has been described in detail.[21]

The use of base-catalysed reactions in elucidating the structures of natural conjugates of uronic acids has been reviewed.[22]

Since the reactions between primary amino-groups and cyclic imidocarbonate derivatives of polysaccharides are considered to yield either *N*-substituted isoureas or carbamates or imidocarbonates, hydrolysis with alkali should lead to the release of amino-compounds.[23] The approach used permitted the released

[14] S. S. Raghavan, R. A. Mumford, and J. N. Kanfer, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 99.
[15] D. Shapiro, A. J. Acher, and Y. Rabinsohn, *Chem. and Phys. Lipids*, 1973, **10**, 28.
[16] J. F. Kennedy, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 305.
[17] J. Turková, *J. Chromatog.*, 1974, **91**, 267.
[18] H. Guilford, *Chem. Soc. Rev.*, 1973, **2**, 249.
[19] C. R. Lowe and P. D. G. Dean, 'Affinity Chromatography', Wiley, Chichester, 1974.
[20] J. F. Kennedy, in 'Connective Tissues; Biochemistry and Pathophysiology', ed. R. Fricke and F. Hartmann, Springer–Verlag, Berlin, 1974, p. 146.
[21] J. F. Kennedy, *Chem. Soc. Rev.*, 1973, **2**, 355.
[22] J. Kiss, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 229.
[23] M. Naoi and Y. C. Lee, *Analyt. Biochem.*, 1974, **57**, 640.

amino-compounds to be measured in the presence of an excess of ammonia that was formed from the unsubstituted carbamate groups on the polysaccharide. Fluorescamine was used to measure non-chromogenic ligands for which specific assays were not available, and 6-aminohexanoic acid and 6-aminohexyl β-D-galactopyranoside were employed as standards. This procedure overcomes the problems associated with determining the amount of ligand attached to a polysaccharide imidocarbonate by difference.

Methods used in determining the structures of polysaccharides have been reviewed.[24]

A new method for the specific degradation of polysaccharides involves methylation to give derivatives containing a limited number of hydroxy-groups at defined positions; following oxidation with ruthenium tetraoxide, the modified polysaccharide was treated with base.[25] Analysis of the elimination products furnished information on the sequence of monosaccharide residues in the polysaccharide.

A review of the ^{1}H n.m.r. spectroscopy of oligo- and poly-saccharides has paid particular attention to ether and acetate derivatives thereof.[26]

Chemical-ionization mass spectroscopy with ammonia and isobutane as the reagent gases has been used to examine the peracetates of several oligosaccharides (DP 2—5).[27] Intense $(M + NH_4)^+$ ions were observed for all but the pentasaccharide acetates, and ions corresponding to the attachment of NH_4^+ ions to fragments produced by thermolysis generally dominated the spectra. The relative importance of thermolysis increased with increasing molecular weight. Chemical-ionization mass spectrometry with ammonia as the reagent gas can be used to determine the molecular weights of acetylated oligosaccharides.

The induced Cotton effects of acid polysaccharide–thiazine complexes have been studied at various temperatures.[28] When the ratio of the anionic sites on the polymer to the cationic dye was near unity, all the complexes showed Cotton effects corresponding to their absorption bands in the visible and ultraviolet regions at neutral pH and room temperature.

Graft copolymerization on to polysaccharides has been discussed in an edited series on block and graft copolymerizations.[29]

Agarose.—Macroporous agaroses in bead form (*e.g.* Sepharose, Bio-Rad Agarose, *etc.*) have been converted into cyclic imidocarbonate derivatives by reaction with cyanogen bromide, and the derivatives were used extensively in the immobilization of biologically active macromolecules and for affinity chromatography. The reaction of the derivatized agarose with the incoming nucleophile is considered to involve attack of amino-groups at the *trans*-imidocarbonate ring to give either an isourea or an N-substituted imidocarbonate or an N-substituted carbonate.

[24] B. Lindberg, in 'Macromolecular Chemistry—8 (Helsinki, 1972)', ed. K. Saarela, Butterworths, London, 1973, p. 231.
[25] L. Kenne, J. Lönngren, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 3692.
[26] M. Vincendon, *Bull. Soc. chim. France*, 1973, 3421.
[27] R. C. Dougherty, J. D. Roberts, W. W. Binkley, O. S. Chizhov, V. I. Kadentsev, and A. A. Solov'yov, *J. Org. Chem.*, 1974, **39**, 451.
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[29] 'Block and Graft Copolymerization', ed. R. J. Ceresa, Wiley Interscience, New York, 1973, Vol. 1.

Recent references to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes) and of 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,[30–39] 2,[40–52] and 3,[53–78] respectively. The derivatives are listed according to the uses

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[42] J. W. Chidlow, F. J. Bourne, and A. J. Bailey, *F.E.B.S. Letters*, 1974, **41**, 248.
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[77] T. Haugen, A. Ishaque, A. K. Chatterjee, and J. Preiss, *F.E.B.S. Letters*, 1974, **42**, 205.
[78] J. D. Oppenheim, M. S. Nachbar, M. R. J. Salton, and F. Aull, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 1127.

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme	*E.C. No.*	*Ref.*
D-Amino-acid oxidase	1.4.3.3	30
α-Amylase	3.2.1.1	31
Glutamate dehydrogenase	1.4.1.2	32
Glycogen phosphorylase	2.4.1.1	33 [a]
Lactate dehydrogenase	1.1.1.27	34
Lactoperoxidase	—	35
Peroxidase	1.11.1.7	35
Phosphodiesterase I	3.1.4.1	36 [b]
endo-Polygalacturonase	— [c]	37
Trypsin	3.4.21.4	38, 39, 39 [d]

[a] Bound in the presence and in the absence of N^6-(6-aminohexyl)adenosine 5′-phosphate; [b] Bound to concanavalin A already coupled to agarose cyclic imidocarbonate; [c] See Chapter 6, p. 377; [d] Bound to a derivative of agarose cyclic imidocarbonate [see (12), p. 415].

Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Anti-α-L-arabinofuranosidase	Immunoadsorption of α-L-arabinofuranosidase	40
Anti-arylsulphatase A	Studies of the binding of human arylsulphatase A to the homologous antibody from other mammals. Enzyme immunoassay for the detection of normal and mutant human arylsulphatases A, and for the detection of metachromatic leukodystrophy	41
Anti-collagen	Isolation of specific collagen peptides	42
Anti-α-foetoprotein	Isolation of α-foetoprotein	43 [a]
Anti-α_1-foetoprotein	Purification of α_1-foetoprotein	44
Anti-β-D-glucuronidase	Purification of β-D-glucuronidase	45
Anti-hepatitis antigen	Removal of hepatitis antigen from blood and plasma	46
Anti-lysozyme	Immunoadsorption and isolation of lysozymes	47
Anti-polygalacturonases	Immunoadsorption of polygalacturonases	40
Collagen	Purification of anti-collagen	42
Goat immunoglobulin anti-β-D-glucuronidase	Purification of β-D-glucuronidase	45
Growth hormones	Purification of anti-(growth hormones), and studies of the immunological properties of immobilized growth hormones	48
	Study of the biological activity of immobilized growth hormone in tissues	49
Malate dehydrogenase (unspecified)	Purification of anti-(malate dehydrogenase) (unspecified)	50
Rabbit immunoglobulin anti-interferon	Purification of interferons	51

Table 2 (*cont.*)

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Rabbit immunoglobulin IgG anti-lysozyme	Immunoadsorption and isolation of lysozymes	47
Soybean agglutinin	Purification of anti-(soybean agglutinin)	52

[a] Using agarose treated with both epichlorohydrin and phloroglucinol prior to treatment with cyanogen bromide.

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials*

Ligand or affinant	*Use of product*	*Ref.*
2-Acetamido-*N*-(6-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine	Purification of β-D-acetamidodeoxyhexosidases A and B	53
Albumin (serum)	Resolution of DL-tryptophan into D- and L-forms	54
N^6-(ω-Aminoalkyl)adenosine monophosphate derivatives (n = 2—10) [a]	Affinity chromatography of D-glucose-6-phosphate dehydrogenase, glyceraldehyde-phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase. Investigations of the effect of n on the efficiency of the matrix	55
N-6-Aminocaproyl β-D-galactopyranosylamine [b]	Isolation of high-molecular-weight, cross-linked agglutinin of soybean	56
6-Aminocaproyl-D-phenylalanine methyl ester [b]	Affinity chromatography of pepsin	57
6-Aminocaproyl-L-phenylalanyl-D-phenylalanine methyl ester [b]	Affinity chromatography of the acid proteinase from *Aspergillus awamori*	57
6-Aminocaproyl-D-tryptophanyl methyl ester [b]	Isolation of chymotrypsin	58
N^6-(6-Aminohexyl)adenosine 5′-monophosphate	Immobilization of glycogen phosphorylase in its active conformation on matrices substituted with an analogue of adenosine 5′-phosphate	33
8-(6-Aminohexyl)amino-adenosine 3′,5′-cyclic phosphate	Purification of adenosine 3′,5′-cyclic phosphate receptor protein and subunits thereof, and the separation of the subunits of protein kinase	59
4-Aminophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside 4-Aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 4-Aminophenyl α-L-fucopyranoside 4-Aminophenyl β-D-galactopyranoside 4-Aminophenyl α-D-mannopyranoside	Affinity chromatography and purification of lectins	60
4-Aminophenylmercuric acid	Isolation of ficin, and the separation of ficin and mercurificin	61

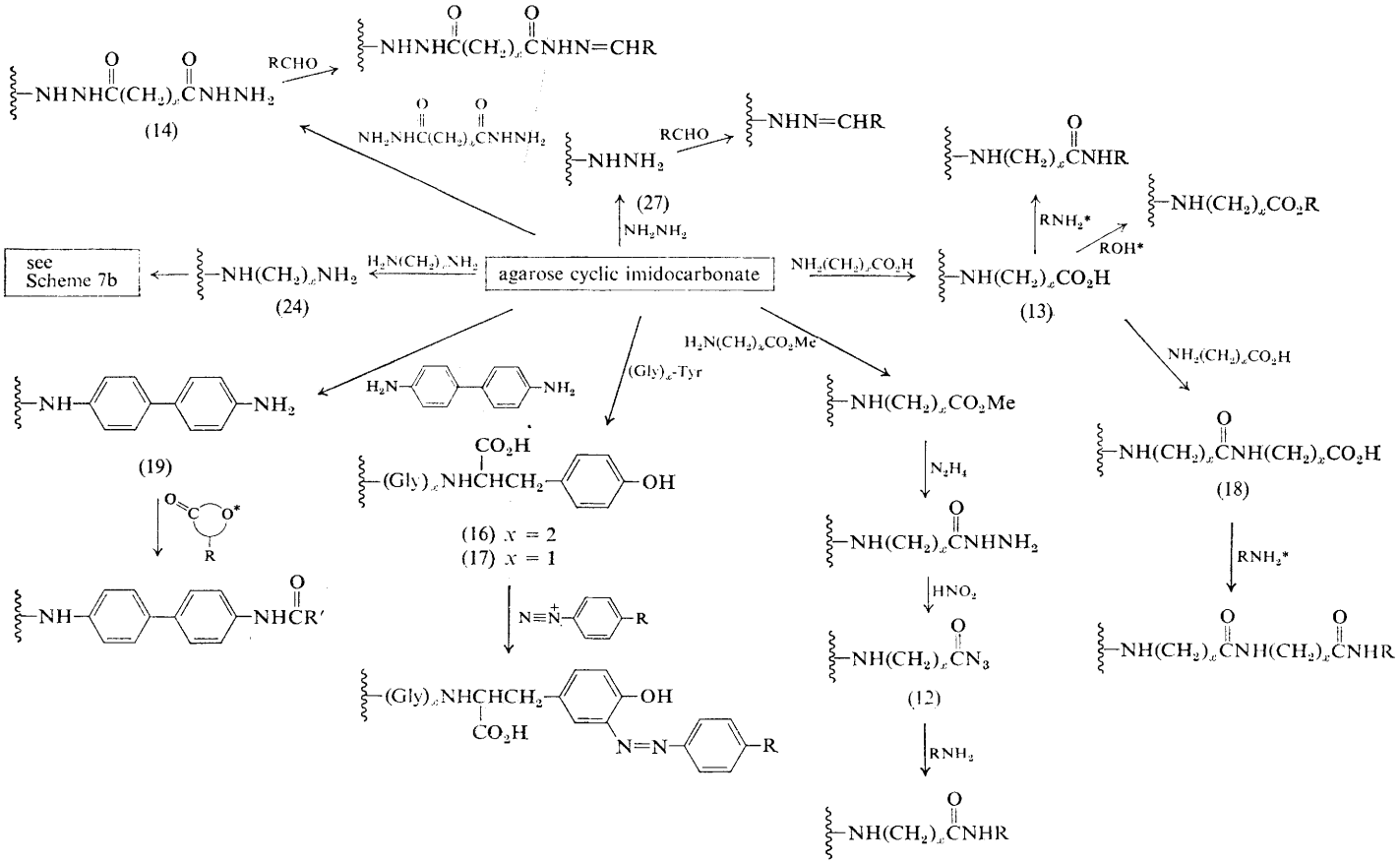
Table 3 (*cont.*)

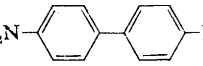
Ligand or affinant	*Use of product*	*Ref.*
5′-(4-Aminophenylphosphoryl)-guanosine 2′(3′)-phosphate 5′-(4-Aminophenyl)uridine 2′(3′)-phosphate	Purification of ribonuclease	62 63
Avidin	Purification of acetyl-CoA apocarboxylase	64
Blue Dextran	Isolation of lactate dehydrogenase	65
Concanavalin A	Affinity chromatography of serum proteins as an assessment of crossed immuno-affinoelectrophoresis	65
	Cross immuno-affinoelectrophoresis	66
	Fractionation of indole-3-acetic acid oxidase-peroxidase into glycoprotein and non-glycoprotein fractions	67
	Isolation of dopamine β-mono-oxygenase	68
	Immobilization of phosphodiesterase I in its active conformation	36
	Separation of erythropoietin from other endogenous glycoproteins	69
1,8-Diamino-octane 1,3-Diaminopropane	Purification of glycerol-3-phosphate dehydrogenase (NAD^+)	70
Gentamicin	Purification of acetyl-CoA acetyltransferase and an unspecified phosphotransferase	71[c]
Glycylglycyl-(*O*-benzyl-L-tyrosyl)-L-arginine	Purification of acrosin	72
α-Lactalbumin	Affinity chromatography of lactose synthetases	73
Neurophysins	Purification of [^{125}I]-labelled L-lysine-vasopressin and the separation of labelled and unlabelled forms thereof	74
Nicotinamide adenine dinucleotide	Affinity chromatography of (nicotinamide adenine dinucleotide)-linked dehydrogenases	75
Ovomucoid	Purification of acetylated, twice acetylated, and succinylated trypsins	76
P′-(6-Phospho-1-hexyl)-*P*^{2}-(6-amino-1-hexyl)-pyrophosphate	Purification of ADP-D-glucose pyrophosphorylase	77
Spermidine	Purification of glycerol-3-phosphate dehydrogenase (NAD^+)	70
Submaxillary mucin glycoprotein (bovine)	Purification of the haemagglutinin from *Limulus polyphemus*	78
Trypsin	Affinity chromatography of trypsin inhibitor	39

[a] Where *n* represents the number of carbon atoms in the linear alkyl chain; [b] The product is identical in structure with that obtained by an alternative method *via* (13) (see p. 415); [c] A mixture of agarose and polyacrylamide beads was employed.

prescribed, since, for example, the insoluble form of an enzyme used for affinity chromatography may not have been enzymically active.

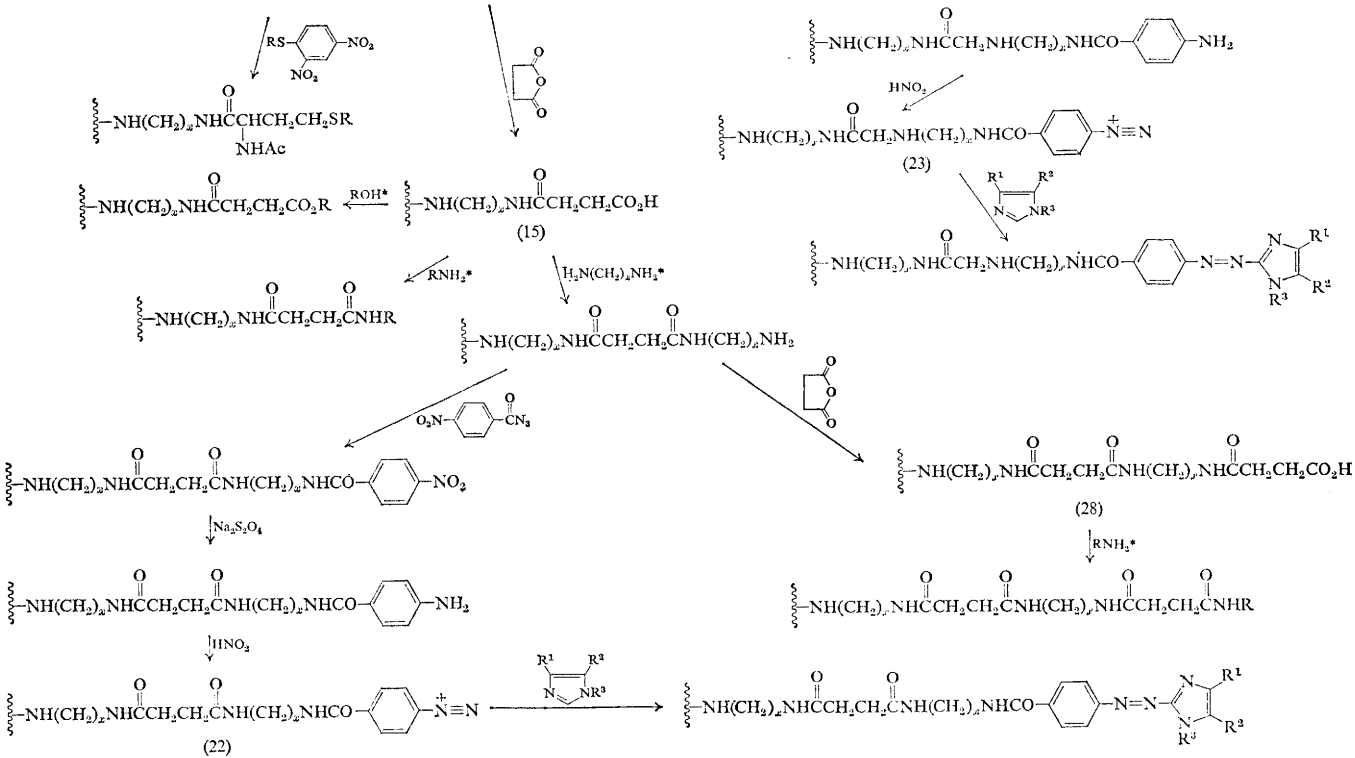
In a simple, reproducible method for the activation of agarose with cyanogen bromide, a solution of the reagent in acetonitrile was added to agarose beads

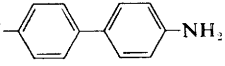


R = rest of affinant, ligand, *etc.* * = reaction involving carbodi-imide. In subsequent references to this scheme, the suffixes A, B, and C denote the use of $H_2N(CH_2)_3NH(CH_2)_3NH_2$, $H_2NCH_2CHOHCH_2NH_2$, and  respectively, in place of $H_2N(CH_2)_xNH_2$ for the production of (24), *etc.*

Scheme 7a

agarose cyclic imidocarbonate
(21)
(29)
(24)
(20)
(25)
(26)



R = rest of affinant, ligand, *etc.* * = reaction involving carbodi-imide. † = another aldehyde can replace acetaldehyde in the reaction. In subsequent references to this scheme, the suffixes A, B, and C denote the use of $H_2N(CH_2)_3NH(CH_2)_3NH_2$, $H_2NCH_2CHOHCH_2NH_2$, and  respectively, in place of $H_2N(CH_2)_xNH_2$ for the production of (24), *etc.*

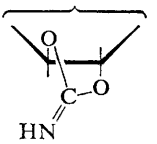
Scheme 7b

suspended in sodium carbonate solution.[79] Titrimetric assay indicated that a reaction time of one minute afforded a product with coupling capacities similar to those of materials produced by conventional methods. The coupling capacities of the products were influenced by the initial concentration of carbonate ion and by the reaction conditions.

A fluorometric method for measuring ligands coupled to agarose cyclic imidocarbonate is based on the reaction of amino-compounds liberated therefrom with fluorescamine.[23] Radioautography has been indicated to be far more sensitive than fluorescent labelling for determining the locale of conjugated species within agarose beads, since it cannot be assumed that low-molecular-weight ligands will be distributed uniformly throughout the bead.[80] Experiments conducted with [^{125}I]-labelled lactoperoxidase and bovine serum albumin provided supporting evidence.

Evidence has been obtained for multi-site attachment in the coupling of poly-(inosinic-cytidylic acids) to agarose cyclic imidocarbonate, and linkages involving the aromatic amino-groups of the polynucleotide were formed.[81] In the reaction of nicotinamide adenine dinucleotide with agarose cyclic imidocarbonate, the bond formed can involve various groups on the ligand.[75]

Direct attachment of an affinity ligand (or enzyme) to the polysaccharide derivative proved to be unrewarding in a number of instances, since the approach of the ligand was sterically impeded. This difficulty has been overcome by inserting a bridge between the matrix and the ligand. Bridging was also useful when functional groups on the ligand did not react with those on the matrix, since it allowed a new type of functionality to be introduced. Reactions used to obtain affinity-chromatography matrices, *etc.* from agarose cyclic imidocarbonates are depicted in Schemes 7a and 7b, and the materials produced are summarized in Table 4.[82–102] All were derived from the structure (30).



(30)

[79] S. C. March, I. Parikh, and P. Cuatrecasas, *Analyt. Biochem.*, 1974, **60**, 149.
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[85] R. G. H. Cotton, *F.E.B.S. Letters*, 1974, **44**, 290.
[86] E. E. Grebner and I. Parikh, *Biochim. Biophys. Acta*, 1974, **350**, 437.
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[88] M. R. Villarejo and I. Zabin, *Nature New Biol.*, 1973, **242**, 50.
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[90] J. I. Rood and R. G. Wilkinson, *Biochim. Biophys. Acta*, 1974, **334**, 168.
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[92] J. N. Kanfer, R. A. Mumford, S. S. Raghavan, and J. Byrd, *Analyt. Biochem.*, 1974, **60**, 200.
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[94] P. O'Carra, S. Barry, and T. Griffin, *F.E.B.S. Letters*, 1974, **43**, 169.
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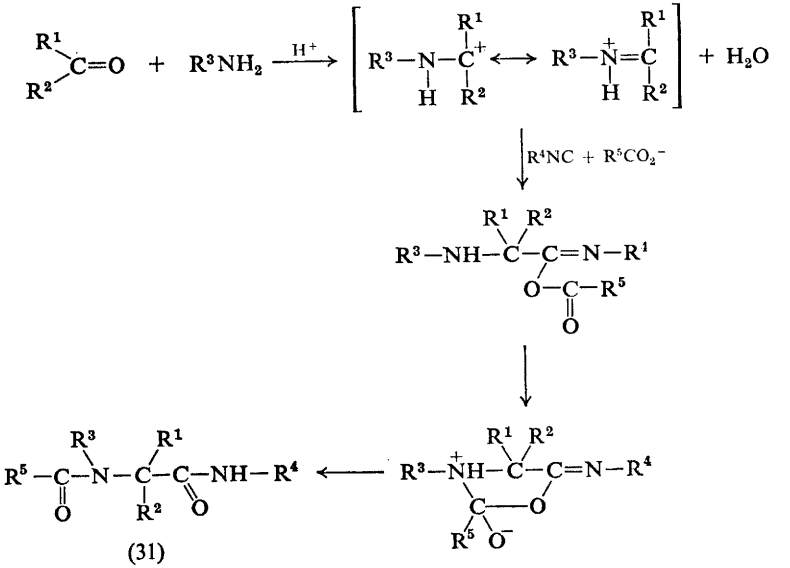
[97] B. H. Anderton, F. W. Hulla, H. Fasold, and H. A. White, *F.E.B.S. Letters*, 1973, **37**, 338.

Agarose cyclic imidocarbonate coupled with 1,6-diaminohexane provides one of the materials (24) most used in linkage extension; the coupling capacity of this material can be ascertained by titration with sodium hydroxide using thymolphthalein as an indicator.[100]

Products obtained by coupling various substances to agarose cyclic imidocarbonates for miscellaneous uses are listed in Table 5.[103–107]

Macroporous agarose has been pre-treated with epichlorohydrin,[103] followed in some cases by reaction with sodium borohydride,[97] prior to activation with cyanogen bromide. Cross-linking of agarose with epichlorohydrin in the presence of phloroglucinol yielded a material containing phloroglucinol attached to the polysaccharide *via* hydroxypropyl groups.[43] All the hydroxy-groups of this material were activated by cyanogen bromide in the production of an immunoadsorbent.

A polymer containing carbonyl groups has been produced by the oxidation of epichlorohydrin-cross-linked and desulphated agarose with DMSO–phosphorus pentaoxide.[103] The product was used to immobilize human serum albumin, chymotrypsinogen A, benzylpenicillin, 6-aminopenicillanic acid, and phenylalanine methyl ester by the reactions shown in Scheme 8. This conversion



Scheme 8

[98] J. J. Befort, N. Befort, G. Pétrissant, P. Remy, and J. P. Ebel, *Biochemie*, 1974, **56**, 625.
[99] R. M. Dawson and H. D. Crone, *J. Chromatog.*, 1974, **92**, 349.
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[105] E. Knight, jun., *Biochem. Biophys. Res. Comm.*, 1974, **56**, 860.
[106] W. A. Frazier, L. F. Boyd, and R. A. Bradshaw, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2931.
[107] B. Lotina, A. Gómez-Puyou, M. Tuenade Gómez-Puyou, and E. Châvez, *Arch. Biochem. Biophys.*, 1974, **159**, 517.

Table 4 *Use of agarose cyclic imidocarbonate for the preparation of affinity-chromatography materials* via *linkage extension*

Ligand or affinant	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Adenosine triphosphate (periodate-oxidized)	(14)	Affinity chromatography of myosin and meromyosin; routine preparation of purified, active myosin species	82
Albumin (serum)	(15)	Resolution of DL-tryptophan into D- and L-forms	54
4-Aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	(15)	Purification of 2-acetamido-2-deoxy-D-glucose-binding proteins	83
2-Amino-2-deoxy-L-fucose	(13)	Purification of α-L-fucosidase	84
2-Amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride	(13)	Purification of phenylalanine 4-mono-oxygenase	85
4-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	(15A)	Purification of β-D-acetamidodeoxyhexosidase A	86
4-Aminophenyl 1-thio-β-D-galactopyranoside	(15)	Purification of β-D-galactosidase	87
	(15A)	Affinity chromatography of β-D-galactosidase and mutant fragments; investigation of the genetic variation of mutant forms for substrate recognition	88
N-(4-diazophenyl)oxamic acid	(16)	Purification of neuraminidase	89
	(17)	Affinity chromatography of haemolysin, neuraminidase, and phospholipase C	90
α-D-Galactopyranosylamine	(18)	Isolation of α-D-galactosidase	91
D-Glucono-1,4-lactone	(19)	Purification of β-D-glucosidase, β-D-glucosylceramidase, and β-D-glucosylsphingosinase	92
6-Mercaptoadenosine monophosphate 6-Mercaptoadenosine triphosphate 6-Mercaptonicotinamide adenine dinucleotide	(20)	Unspecified affinity chromatography	93
Nicotinamide adenine dinucleotide and analogues and derivatives thereof	(13), (15), (21), (22)	Affinity chromatography of (nicotinamide adenine dinucleotide)-linked dehydrogenases	75
	(20), (20B), (21), (21B), (23B)	Investigation of spacer arms in affinity chromatography; use of hydrophilic spacer arms to control or eliminate non-biospecific adsorption effects; affinity chromatography of (nicotinamide adenine dinucleotide)-linked enzymes	94

19-Nortestosterone-17-*O*-hemisuccinate (radioactive)	(24A)	Purification of steroid Δ-isomerase	95
Oxalic acid	(24), (24B)	Differentiation of lactate dehydrogenase isoenzymes	96
	(25B)	Investigation of spacer arms in affinity chromatography; use of hydrophilic spacer arms to control or eliminate non-biospecific adsorption effects; affinity chromatography of (nicotinamide adenine dinucleotide)-linked enzymes	94
6-(Purine 5′-ribosyltriphosphate)-4-(1,3-dinitrophenyl) thioether	(26A)	As an adenosine triphosphate analogue for the purification of adenosine triphosphatase	97 [b]
t-Ribonucleic acid (periodate-oxidized)	(27)	Purification of aminoacyl-tRNA synthetases, including seryl-tRNA synthetase	98
Tri-[^{14}C]methyl-(3-aminophenyl)ammonium chloride hydrochloride Tri-[^{14}C]methyl-(4-aminophenyl)ammonium chloride hydrochloride	(28A) [c]	Purification of acetylcholinesterase	99
Trypsin	(12)	Affinity chromatography of trypsin inhibitor	39
2,4,6-Trinitrobenzenesulphonic acid	(24)	Isolation of glycerol-3-phosphate dehydrogenase (NAD^+)	100
L-Tryptophan methyl ester	(13)	Affinity chromatography of chymotrypsin and the separation of chymotrypsin from derivatives thereof	101
Vitamin B_{12}—monocarboxy-derivatives thereof	(24A)	Purification of gastric mucosal intrinsic and non-intrinsic factors	102

[a] See Scheme 7; [b] Agarose was pre-treated with epichlorohydrin and sodium borohydride prior to reaction with cyanogen bromide; [c] A in second diamino-reaction only.

Table 5 *Use of agarose cyclic imidocarbonates for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
No further substitution	—	Experimental control of the affinity chromatography of haemagglutinins	60
Acetylated derivative of *N*-hydroxysuccinimide	(24) and (25)	Control gels in investigations of spacer arms in affinity chromatography and in the use of hydrophilic spacer arms to control or eliminate non-biospecific adsorption effects	94
Adenosine 5′-phosphate	(29C) [b]	Investigation of the use of isocyanides for the immobilization of biological molecules	103
L-[^{14}C]alanine	—	Testing of a method for the conversion of agarose into cyclic imidocarbonate derivatives using cyanogen bromide	79
Albumin (human serum)	(29C) [b]	Investigation of the use of isocyanides for the immobilization of biological molecules	103
Albumin (serum)	—	Experimental control in studies of the bactericidal effectiveness of immobilized peroxidases	35
[^{125}I]-Albumin (serum)	—	Determination of the binding of proteins to agarose cyclic imidocarbonate by auto-radiography	80
	—	Testing of a method for the conversion of agarose into cyclic imidocarbonate derivatives using cyanogen bromide	79
6-Aminohexanoic acid	—	Experimental control in the affinity chromatography of chymotrypsin	101
	—	Experimental control in the affinity chromatography of phenylalanine 4-mono-oxygenase	85
	—	As a standard for fluorometric measurement of ligands incorporated into agarose cyclic imido-carbonate	23
6-Aminohexyl β-D-galactopyranoside	—	As a standard for fluorometric measurement of ligands incorporated into agarose cyclic imido-carbonate	23
6-Aminopenicillanic acid Chymotrypsin	(29C) [b]	Investigation of the use of isocyanides for the immobilization of biological molecules	103

Concanavalin A	—	Investigation of the binding of β-D-galactosidases and β-D-glucosidase to plant haemagglutinins	104
1,6-Diaminohexane	—	Experimental control in the affinity chromatography of β-acetamidodeoxy-D-glucosidase, β-D-galactosidase, and α-D-mannosidase	83
N-(4-Diazophenyl)oxamic acid	—	Positive experimental control in the affinity chromatography of haemolysin, neuraminidase, and phospholipase C	90
L-Fucose-binding protein from *Lotus tetraglobinus*	—	Investigations of the binding of β-D-galactosidases to plant haemagglutinins	104
Guanosine 2′(3′)-phosphate	—	Unsuccessful trial in the purification of ribonucleases	62
Heme proteinoid	—	Experimental control in studies of the bactericidal effectiveness of immobilized peroxidases	35
Interferon	—	Induction of resistance to virus replication in the L-cells of mice	105
[^{125}I]Lactoperoxidase	—	Determination of the binding of proteins to agarose cyclic imidocarbonate by autoradiography	80
Nerve growth factor	—	Investigations of the biological interactions of immobilized nerve-growth factor	106
Nicotinamide adenine dinucleotide Poly(adenylic acid)	(29C) [b]	Investigation of the use of isocyanides for the immobilization of biological molecules	103
Poly(cytidylic acid) Poly(inosinic acid)	— —	Induction of interferon stimulation	81
Ribonucleic acid	(29C) [b]	Investigation of the use of isocyanides for the immobilization of biological molecules	103
Succinic anhydride	(24)	Experimental control in the affinity chromatography of β-acetamidodeoxy-D-glucosidase, β-D-galactosidase, and α-D-mannosidase	83
	(24)	Experimental control in the affinity chromatography of D- and L-tryptophan	54
Synthatin (decamethylene diguanidine)	—	Investigation of the inhibition of mitochondrial respiration by immobilized synthatin	107
Wheat-germ agglutinin	—	Investigation of the binding of β-D-galactosidases to plant haemagglutinins	104

[a] See Scheme 7b, pp. 416 and 417; [b] The agarose beads were cross-linked with epichlorohydrin prior to conversion into the cyclic imidocarbonate.

proceeded in aqueous solution under physiological conditions to give products exemplified by (31), and was used to evaluate isocyanides as reagents for the immobilization of biological molecules.

Agarose derivatized with concanavalin A has been used in the separation of antigens induced by Herpes simplex virus[108] and for the immobilization of phosphodiesterase I; the product in the latter case was used to hydrolyse poly-(adenylic acid), *etc.*[36]

Unmodified agarose has been used in the purification of glycerol 3-phosphate dehydrogenase(NAD^+).[70]

Alginic Acid.—Trimethylsilylated derivatives were obtained when alginic acid and a propylene glycol ester thereof were heated in formamide with an excess of hexamethyldisilazane.[109] On completion of the reaction, the trimethylsilylated derivatives were precipitated with acetone. This route avoided contamination of the products with ammonium chloride.

The degradation of carbohydrates containing uronic acid residues by β-elimination has been reviewed.[110]

Amyloses.—Amylose has been proposed as a model for isotactic polymers.[111] The preparation of derivatives of amylose was discussed, and the results of an investigation of amylose carbanilates by light scattering and electron microscopy were reported.

^{1}H N.m.r. spectra of the reducing end-group of such amylose derivatives as the acetate have been used to determine the specificities of reactions involving these groups, *e.g.* formation of the α-bromide.[112]

The behaviour of amylose acetate in dilute solutions of nitromethane has been reassessed using linear fractions of low molecular weight.[113] The results indicated that amylose acetate assumes a stiff-coil conformation in good solvents.

6-*O*-Tritylamylose (32) has been converted into a glycan (33) containing 2-amino-2-deoxy-D-glucosyl residues and some *O*-(methylthio)methyl groups (Scheme 9).[114] Removal of the ether groups gave a 2-amino-2-deoxyamylose (34) of d.s. 0.54 that was cleaved by α-amylase to oligosaccharides containing amino-sugar residues. Further transformations on (33) afforded the 2-amino-2-deoxy-α-D-glucopyranuronan (35) (d.s. by amine of up to 0.65 and by carboxy of 0.46). Sulphation of (35) gave a (1 → 4)-2-deoxy-2-sulphoamino-α-D-glucopyranuronan [isolated as its sodium salt (36)] that showed appreciable blood-anticoagulant activity.

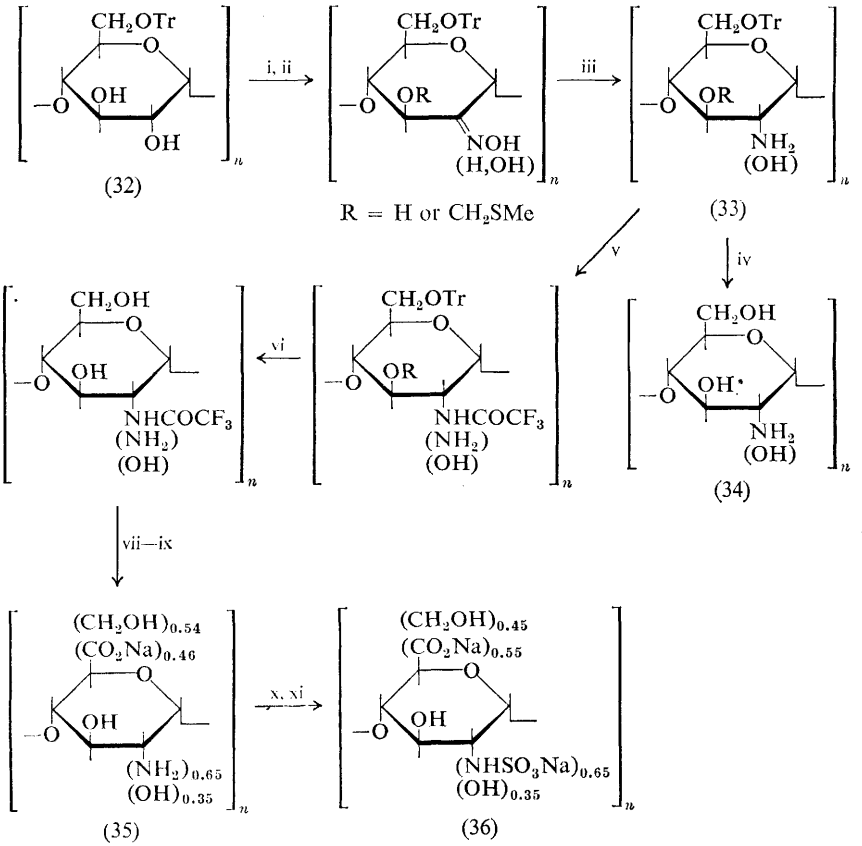
The interaction of amylose with borate anions has been analysed by frontal gel filtration using only the constituent velocity data.[115] The constituent velocity equation was reformulated in terms of the elution volume for a system described

[108] M. Ponce de Leon, H. Hessle, and G. H. Cohen, *J. Virol.*, 1973, **12**, 766.
[109] R. E. Harmon, K. K. De, and S. K. Gupta, *Carbohydrate Res.*, 1973, **31**, 407.
[110] J. Kiss, *Adv. Carbohydrate Chem. Biochem.*, 1974, **29**, 229.
[111] E. Husemann, in 'Macromolecular Chemistry—8 (Helsinki, 1972)', ed. K. Saarela, Butterworths, London, 1973, p. 85.
[112] D. Gagnaire and M. Vincendon, *Carbohydrate Res.*, 1973, **31**, 367.
[113] J. M. G. Cowie and A. Maconnachie, *European Polymer. J.*, 1975, **11**, 79.
[114] D. Horton and E. K. Just, *Carbohydrate Res.*, 1973, **30**, 349.
[115] M. Janado, J. Azuma, and K. Onodera, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2337.

by the equation:

$$A + iB = ABi \quad (i = 1, 2, 3, \ldots, n)$$

Examination of the binding data indicated that this type of equilibrium operates predominantly, if not solely, in the formation of complexes between amylose and borate anions. This approach enabled the association constant and the number of binding sites to be evaluated, but no evidence was obtained for the formation of inclusion complexes.



Reagents: i, DMSO–Ac_2O; ii, NH_2OH–py; iii, $LiAlH_4$–THF; iv, HCl–EtOAc; v, $(CF_3CO)_2O$–py; vi, HCl–$CHCl_3$; vii, O_2–Pt; viii, H^+; ix, HO^-; x, SO_3–py; xi, $ClSO_3H$–py

Scheme 9

Carboxymethylated, phenylacetylated, tosylated, succinylated, and phthalylated derivatives of amylose have been prepared.[116] The glass-transition (T_g) and melting (T_m) temperatures of these derivatives and of amylose acetate, propionate, palmitate, and benzoate were measured; both T_g and T_m decreased with increasing

[116] D. H. Desai, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1973, **25**, 162.

chain-length of the pendant group. The average value (*ca.* 0.8) of $T_g : T_m$ showed that the esters are moderately flexible, unsymmetric polymers. The heat of fusion for the aliphatic esters decreased as the chain-length of the pendant group increased.

Activity coefficients have been measured for uni- and bi-valent cations interacting with such polysaccharides as carboxymethylamylose.[117] The structure of the polysaccharide did not appear to influence specifically the values of the activity coefficients. The cation selectivities exhibited by the polysaccharide derivatives were discussed in terms of the selectivity coefficient (K_{Na}^{Ca}) and the free energy of exchange (ΔG_{Na}^{Ca}); ΔG_{Na}^{Ca} is related to the charge density, whereas K_{Na}^{Ca} is sensitive to the structure of the chain. Carboxymethylamyloses were highly specific for certain cations. The pK_0 values of such polysaccharide derivatives were practically independent of the nature of the polyelectrolyte and of the charge density; the values were close to those of the monomers and were unaffected by the position of the carboxy-group on the D-glucose ring. In conjunction with a theoretical approach, these studies also confirmed the rigidity of the polysaccharide chains.

Viscometry, osmometry, and light-scattering techniques have been used to study the properties of amylose propionate in solution, and the relations between the intrinsic viscosity, weight-average molecular weight, radius of gyration, and second virial coefficient were established.[118] Several theories of viscosity were used to calculate the unperturbed dimensions of the polymer molecules in solution. The hydrodynamic behaviour indicated that the polymer assumes a flexible-coil configuration in solution. The stiffness of the polymer chains may be due to steric repulsions between neighbouring substituents. A number of parameters have been measured for dilute solutions of linear amylose propionate in ethyl acetate and THF.[119] Mark–Houwink relationships were established for the molecular-weight range $1—15 \times 10^4$, and the results indicated that the coil is only slightly expanded in these solvents. The characteristic ratio C_∞ was calculated to be 6.21, leading to a steric parameter of $\sigma = 2.32$, while $d\ln C_\infty/dT$ was found to be negative.

Short-chain amylose (DP = 20) with a reduced terminal unit has been used as the substrate in an automated method for determining specific glucanases in the presence of pullulanase and other debranching enzymes.[120]

Amylose has been grafted to polystyrene using Fe^{II} ions and hydrogen peroxide to initiate the process.[121] By evaluation of the chain-transfer constant and kinetic data, conditions that caused a decrease in the extent of the termination of homopolymer and graft-copolymer radicals, but that showed an increase in the yield of the graft copolymer, were assessed. The grafted material was acetylated, and graft fractions containing different proportions of styrene were studied by light scattering, viscosity, and osmometry.[122] The experimental results were compatible with predictions based on physicochemical theory of branched copolymers.

[117] M. Rinaudo and M. Milas, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1974, **12**, 2073.
[118] R. A. Thakker, C. K. Patel, and R. D. Patel, *Stärke*, 1974, **26**, 9.
[119] J. M. G. Cowie and A. Maconnachie, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1974, **12**, 407.
[120] K. Kainuma, K. Wako, A. Nogami, and S. Suzuki, *J. Jap. Soc. Starch. Sci.*, 1973, **20**, 112.
[121] C. M. Patel, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1973, **25**, 233.
[122] C. M. Patel and V. M. Patel, *Stärke*, 1973, **25**, 136.

Carrageenan.—Chloroquine has been shown to interact with carrageenan since it prevented swelling in rats produced by injection of carrageenan.[123] The interaction is pH-dependent, and the resulting complex is soluble in saline. Theories of complex formation were applied to the accumulation of chloroquine that can occur in connective tissues.

Cellulose.—The mol.-wt. distribution of celluloses degraded by methanolysis has been studied by gel-permeation chromatography.[124] The accessible portion of the sample appeared to be degraded preferentially, yielding fragments with an average DP of 8. The results were interpreted in terms of 'weak' linkages along the cellulosic chains.

The effects of crystallinity, orientation, and degree of polymerization on the pyrolyses of cellulose *in vacuo* and in air have been investigated.[125] In contrast to the pyrolysis *in vacuo*, which was interpreted in terms of an uncatalysed reaction, that in air was interpreted in terms of an oxygen-catalysed reaction.[126] The formation of laevoglucosan proved to be a function of the crystallinity and orientation of a particular cellulose, and a proportion of laevoglucosan appeared to be formed in less-ordered regions. Orientational effects on the pyrolysis of cellulose have been investigated with a series of rayon fibres differing only in orientation.[127] The initial loss of weight during low-temperature, vacuum pyrolyses resulted from cross-linking in the less-ordered regions. The extent of this first-order reaction decreased with increasing orientation and was directly related to the amount of charring at higher temperatures.

Cellulose radicals produced by photo-irradiation have been studied by e.s.r. spectroscopy.[128] Single-line spectra were observed initially, but additional lines appeared later, particularly when sensitizers (*e.g.* hydrogen peroxide or metal ions) were used. It was suggested that cellulose radicals giving rise to single-line spectra were most important in initiating graft copolymerizations.

Cellulose fibres have been shown to nucleate preferentially the crystallization of polypropylene; the effect of the form of cellulose on the nucleation was investigated.[129]

Cellulose has been proposed as a model for syndiotactic polymers, and the preparation of derivatives of cellulose was discussed.[111] Relations between the morphology and chain-packing of acetylated and methylated celluloses were considered, and investigations of cellulose tricarbanilates by light scattering and electron microscopy were also reported.

Cellulose acetate (d.s. 2.45) has been examined by light scattering, osmometry, and viscometry in three solvents.[130] The data gave the dependences of the mean-square radius of gyration (r), the second virial coefficient (v), and the intrinsic viscosity (η) on the molecular weight (M) and temperature (T). The results showed that excluded-volume effects on r are negligible, even if v is large and

[123] K. F. Swingle, *Biochem. Pharmacol.*, 1974, **23**, 1669.
[124] R. St. J. Manley. *J. Polymer Sci., Part A-2, Polymer Phys.*, 1974, **12**, 1347.
[125] A. Basch and M. Lewin, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1973, **11**, 3071.
[126] A. Basch and M. Lewin, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1973, **11**, 3095.
[127] A. Basch and M. Lewin, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1974, **12**, 2053.
[128] Y. Ogiwara and H. Kubota, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1973, **11**, 3243.
[129] D. G. Gray, *J. Polymer Sci., Part B, Polymer Letters*, 1974, **12**, 509.
[130] D. W. Tanner and G. C. Berry, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1974, **12**, 941.

dv/dT is positive, and the large value found for $d\ln\eta/d\ln M$ was ascribed to partial draining effects. Similar data for other cellulose esters in the literature were also interpreted, and the aggregation of cellulose acetate in many solvents was discussed.

The amorphous nature of cellulose acetate precipitated from a solution of methylene chloride–methanol has been confirmed by *X*-ray analysis.[131] Crystalline and amorphous cellulose acetates were degraded with sulphuric acid in methanol and saponified with sodium methoxide prior to nitration with a mixture of phosphorus pentaoxide and fuming nitric acid. The distribution of chain-lengths of the nitrated materials was determined by gel-permeation chromatography. Comparison of the data was made to ascertain whether the chromatograms exhibited multiple peaks consistent with chain-folding of the degraded materials.

Changes in morphology of the fibres during the transformation of cellulose acetate I into cellulose acetate II have been investigated.[132] The kinetics of evaporation of acetone from films cast from cellulose acetate, acetone, water, and an inorganic additive have been analysed.[133] Criteria for optimizing the performance of the membrane, with respect to the thickness of the film, the casting temperature, the swelling or gelling properties, and the characteristics of the inorganic additive, were also investigated.

^{1}H N.m.r. spectroscopy of cellulose derivatives has been used to determine the specificity of reactions involving the reducing-end groups; *e.g.* the selective formation of the α-bromide when cellulose triacetate reacted with hydrobromic acid in acetic acid.[112] The α-bromide yielded the β-glycoside with 2-methylpropan-2-ol in the presence of silver carbonate. Cellulose nitrate was also prepared.

Cellulose acetates have been modified using antimicrobial agents.[134] The effects of heat, light, and storage, *etc.* on the modifications were examined.

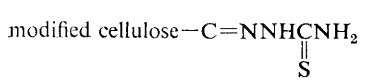
β-D-Fructofuranosidase has been immobilized with cellulose acetate in a fibre-entrapped form.[135]

X-Ray diffraction has been used to investigate the effects of catalysts, the chain-length of the acyl groups, and the degree of esterification on the abilities of partly acylated celluloses to crystallize.[136] The propensity to crystallize was related to the distribution of acylated units in the macromolecules. Acetylation using an acid catalyst appeared to yield blocks of acetyl groups, whereas acylation using a basic catalyst gave a random or more-homogeneous distribution of groups throughout the entire fibre structure. The crystallizability improved with increasing chain-length of the acyl substituent.

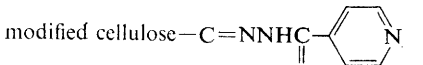
Methods for determining the total content of carbonyl groups in celluloses have been reported.[137]

[131] R. St. J. Manley, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1973, **11**, 2303.
[132] H. D. Chanzy and E. J. Roche, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1974, **12**, 2583.
[133] J. Vinit, J. L. Halary, C. Noel, and L. Monnerie, *European Polymer J.*, 1975, **11**, 71.
[134] A. P. Paulauskas, L. V. Yasyukyavichyute, A. L. Kazilyunas, R. B. Alexandravichyus, A. Y. Kazakyavichyus, L. A. Vole, B. O. Polischyuk, and V. V. Kotetskiy, *Cellulose Chem. Technol.*, 1973, **7**, 417.
[135] W. Marconi, S. Gulinelli, and F. Morisi, *Biotechnol. and Bioeng.*, 1974, **16**, 501.
[136] E. A. Abramova, L. A. Volkova, Yu. A. Meltser, and S. N. Danilov, *Doklady Akad. Nauk S.S.S.R.*, 1974, **215**, 335.
[137] M. Lewin, *Methods Carbohydrate Chem.*, 1972, **6**, 76.

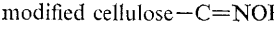
Dialdehydocellulose (obtained by periodate oxidation) reacted with thiosemicarbazide, isonicotinic acid hydrazide, and hydroxylamine hydrochloride to give (37), (38), and (39), respectively.[138] The ability of these polymers to chelate a series of metal ions, and the reaction times and equilibria were investigated. All the derivatives were selective for Cu^{II} and Hg^{II} ions; (37) was also selective for



(37)



(38)



(39)

Ag^{I} and Pb^{II} ions, and (39) for Ag^{I} ions. The polymers removed 83—100% of Cu^{II} ions from aqueous solutions containing equimolar quantities of Cu^{II} and Mg^{II} ions. The amount of Cu^{II} ions combined depended on the pH, and the amounts of H^{+} ions liberated were in linear relationship with the amounts of Cu^{II} ions bound with (37) and (38) but not with (39).

The accelerated ageing of 6-aldehydocellulose in dry and humid conditions resulted in rapid and extensive coloration, as measured by the decrease in the reflectance.[139] 6-Aldehydocellulose was hydrolysed with acid more readily than was cellulose.

DMSO-acetic anhydride oxidized a number of the hydroxy-groups of cellulose to aldehydo- and keto-groups, while other hydroxy-groups were transformed into (methylthio)methyl ethers.[140] The extent of oxidation and etherification was influenced by the ratio of the two reagents and by the reaction conditions. The total carbonyl content of the oxidized cellulose was determined by conversion into the polyoxime, whilst the content of aldehydo-groups was determined following further oxidation with chlorous acid and measurement of the carboxy-groups formed by a spectrophotometric method involving the adsorption of Methylene Blue.

Aminoethylated cellulose has been used for the immobilization of β-D-galactosidase, with retention of activity; the enzyme was coupled with the aid of glutaraldehyde.[141]

The effects of gases on changes in the degree of polymerization of alkali cellulose on heating have been investigated.[142] Acetylated and cyanoethylated derivatives of partly benzylated celluloses were prepared, and the rate of formation of these derivatives was investigated as a function of the degree of substitution by the benzyl ether. Mixed acetylated and cyanoethylated benzyl ethers of cellulose were characterized by the strength of films produced therefrom. The effects of the nature and extent of substitution on the phase-aggregate state and on the

[138] T. Koshijima, R. Tanaka, E. Muraki, A. Yamada, and F. Yaku, *Cellulose Chem. Technol.*, 1973, **7**, 197.
[139] A. E. Luetzow and O. Theander, *Svensk Papperstidn.*, 1974, **77**, 312.
[140] S. L. Snyder, T. L. Vigo, and C. M. Welch, *Carbohydrate Res.*, 1974, **34**, 91.
[141] D. L. Regan, P. Dunnill, and M. D. Lilly, *Biotechnol. and Bioeng.*, 1974, **16**, 333.
[142] E. A. Plisko, A. K. Khripunov, and V. A. Gherasimova, *Cellulose Chem. Technol.*, 1973, **7**, 425.

thermal and thermomechanical properties of the cellulose derivatives were also investigated.[143] Structural changes in partly benzylated celluloses and in the derivatives therefrom were followed by *X*-ray diffraction analysis.

Bromoacetylcellulose reacted with the product resulting from treatment of a serum albumin with hydroxocobalamin in the presence of a carbodi-imide.[144] The complex was used in the purification of human intrinsic factor (cobalamin-binding proteins) by affinity chromatography.

Electro-optical, dynamo-optical, and hydrodynamic properties of solutions of cellulose carbanilate in *p*-dioxan have been investigated.[145] Strong dispersion of the Kerr effect was observed in a variable electric field, indicating that a dipole-orientation mechanism is responsible for electrical birefringence and for its relaxation. Comparison of the relaxation times with the molecular weights and intrinsic viscosities of the fractions showed that rotation of the whole molecule in an electric field is responsible for the Kerr effect. The dependence of the relaxation time on the molecular weight showed that the conformation of cellulose carbanilate changes from a slightly curved rod to a rigid Gaussian coil with increasing molecular weight. In the Gaussian range, the Kerr effect depended on the longitudinal component of the dipole moment formed by the C—O bonds in the D-glucose ring, whereas transverse components of the monomer dipoles began to play an important part in birefringence with low-molecular-weight materials.

Carboxymethyl-, hydroxyethyl-, hydroxypropyl-, and methyl-celluloses have been prepared by reaction of the polysaccharide with sodium monochloroacetate, ethylene oxide, propylene oxide, and methyl chloride, respectively.[146] *X*-Ray analysis of the samples suggested that the substituents are distributed uniformly along the chains. A two-dimensional lattice structure was proposed in which the 101 plane is virtually undisturbed by alkylation, even though the attached groups lie in this plane. Consequently there is an extension of the interplanar d_{101} distances and decrystallization of the 101 plane.

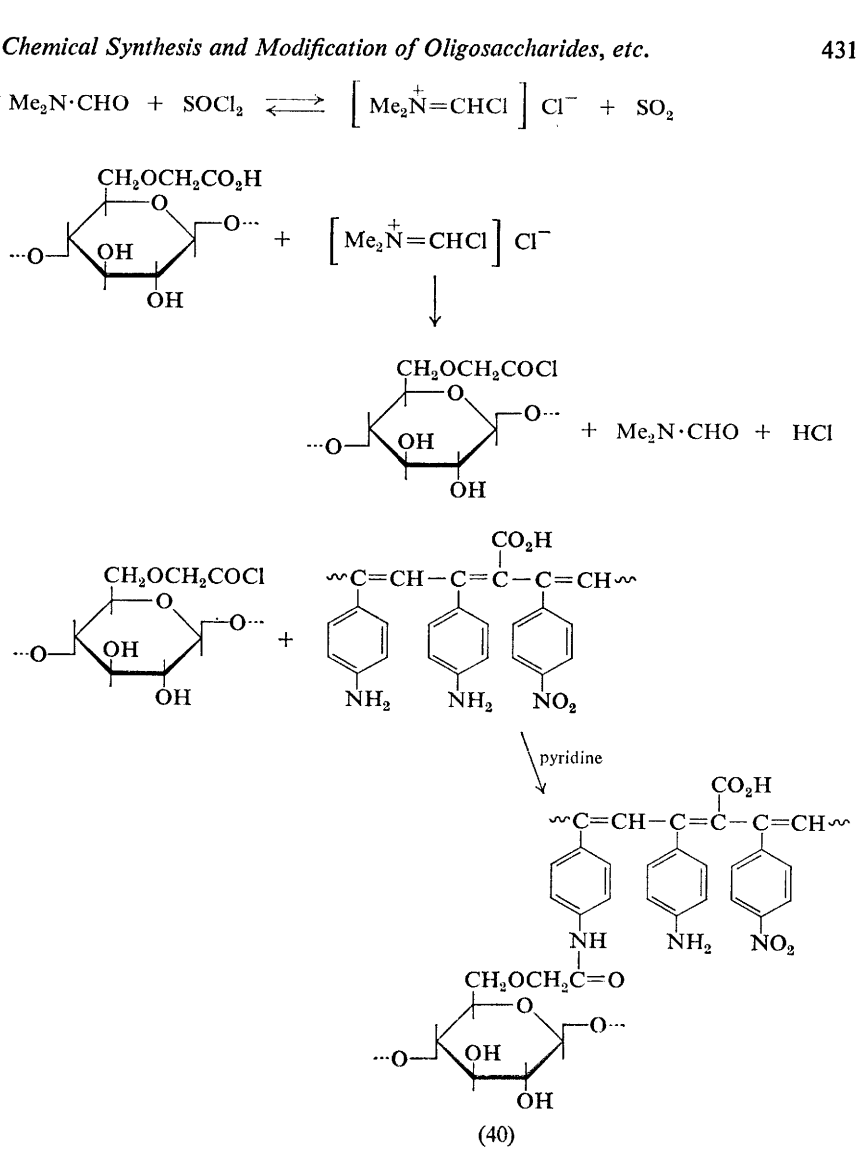
A study of the Cotton effects induced in carboxymethylcellulose by complexation with thiazine showed that increases in temperature gradually decreased the metachromatic shift in the absorption spectrum and the amplitude of the Cotton effects.[28]

Activity coefficients have been measured for uni- and bi-valent cations interacting with carboxymethylcellulose.[117]

Highly active derivatives were obtained when chymotrypsin and a glutamate dehydrogenase were attached to carboxymethylcellulose by means of carbodi-imide.[147]

A mechanism (Scheme 10) has been suggested for the conversion of carboxymethylcellulose into the corresponding acid chloride following treatment with

[143] E. A. Plisko, Y. G. Baklaghina, S. K. Zakharov, Y. N. Sazanov, N. Y. Dolotova, G. N. Fedorova, and V. A. Gherasimova, *Cellulose Chem. Technol.*, 1973, **7**, 433.
[144] J. M. Christensen, E. Hippe, H. Oleson, M. Rye, E. Haber, L. Lee, and J. Thomsen, *Biochim. Biophys. Acta*, 1973, **303**, 319.
[145] V. N. Tsvetkov, E. I. Rjumtsev, L. N. Andreeva, N. V. Pogodina, P. N. Lavrenko, and L. I. Kutsenko, *European Polymer J.*, 1974, **10**, 563.
[146] B. V. Vasiliev, G. I. Bleivas, and M. V. Prokofieva, *Cellulose Chem. Technol.*, 1973, **7**, 293.
[147] I. Mezzasoma and C. Turano, *Boll. Soc. ital. Biol. sper.*, 1971, **47**, 407.



Scheme 10

DMF and thionyl chloride.[148] The acid chloride reacted with a partly reduced poly(4-nitrophenylacetylene) to give (40), the thermal stability of which was investigated.

A study has been made of the ion-exchange sorption by monocarboxycellulose of uni- and bi-valent cations from aqueous solutions of their nitrates.[149] The

[148] C. Simionescu, S. Dumitriu, V. Perec, and S. Dumitrescu, *Cellulose Chem. Technol.*, 1973, **7**, 283.

[149] V. E. Kaputskii, F. N. Kaputskii, S. A. Mechkovskii, V. S. Soldatov, G. D. Zakrevskaya, A. I. Shirko, and V. A. Bredelev, *Kolloid Zhur.*, 1974, **36**, 43.

greater selectivity of the polysaccharide derivative, compared to other absorbents, was attributed to its strongly acidic character.

Native cotton yarn reacted rapidly with phosphoryl chloride in DMF to furnish highly chlorinated (d.s. $\geqslant 0.5$) and phosphorylated celluloses and cellulose formate.[150] The rates of chlorination and phosphorylation could be controlled by varying the concentration of phosphoryl chloride; the effects of the reaction conditions on the formation, tensile strength, and flammability of the products were also investigated. Yarns of high chlorine content were highly tensile, while those of high phosphorus content were flame resistant. Mechanisms were advanced for the chlorination and phosphorylation processes.

Cellulose has been esterified with keten dimer to give (41).[151] The amount of dimer attached was determined, and the process was used in the treatment of paper.

cellulose-$OCOCH_2COMe$

(41)

NN-Diethylaziridinium chloride has been used to assess the availability and disposition of hydroxy-groups on the surfaces of crystalline cellulose.[152] Whereas all the hydroxy-groups in disordered cellulose were found to be equally available, the relative availability of the hydroxy-groups at C-2, C-3, and C-6 on the surfaces of crystalline cellulose was 1.0 : 0.26 : 0.72, respectively, compared with estimated values of 1.0 : 0.0 : 0.075 for those on the surfaces of elementary fibrils of an idealized, perfectly ordered cellulose. Further studies of this reaction have been reported.[153]

The reaction between ethyl chloride and the hydroxy-groups of dissolved and undissolved fibres of cellulose in aqueous alkali has been studied.[154] In each case, substitution at HO-3 was much slower than that at HO-2 or -6. The rate of reaction for the dissolved fibres was proportional, within certain limits, to the concentration of hydroxide ions, whereas the ratios (7 : 1 : 6.5) of the rate constants for HO-2, -3, and -6 of the undissolved fibres were virtually independent of the concentration of hydroxide ions.

The intensity of light scattered from dilute solutions of cellulose nitrate (molecular weight 4.6×10^5) in acetone has been measured both in the absence and in the presence of a.c. and d.c. fields.[155] Due to a mathematical error and a false interpretation in an earlier study, other workers have interpreted similar measurements in terms of a freely rotating, polar chain model. Now it has been demonstrated that cellulose nitrate is better regarded as being extremely stiff and extended. A homodyne light-beat spectrometer equipped with an argon-ion laser and a correlator has been used in a study of the behaviour of the thread-like molecules of cellulose nitrate in dilute solution.[156] This technique permitted

[150] T. L. Vigo and C. M. Welch, *Carbohydrate Res.*, 1974, **32**, 331.
[151] M. Enke, *Cellulose Chem. Technol.*, 1973, **7**, 487.
[152] S. P. Rowland, E. J. Roberts, and A. D. French, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1974, **12**, 445.
[153] S. P. Rowland and E. J. Roberts, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1974, **12**, 2099.
[154] O. Ramnas, *Acta Chem. Scand.*, 1973, **27**, 3139.
[155] B. R. Jennings and J. F. Schweitzer, *European Polymer J.*, 1974, **10**, 459.
[156] G. Meyerhoff and G. Büldt, *Makromol. Chem.*, 1974, **175**, 675.

the translational diffusion coefficient of the molecules to be measured more easily than by conventional methods.

The sulphation of cellulose with chlorosulphonic acid, the pyridine–sulphur trioxide complex, and concentrated sulphuric acid in the presence of various diluents has been studied.[157] The highest degree of sulphation (d.s. 2.0) was obtained using concentrated sulphuric acid in n-butanol containing an amount of water equivalent to the amount of cellulose used.

A trimethylsilylated derivative of cellulose has been prepared by treating a solution of the polysaccharide in formamide with an excess of hexamethyldisilazane.[109]

Cellulose impregnated with chymotrypsin has been cross-linked with glutaraldehyde to produce an active, immobilized enzyme.[158]

Application of classical methods of grafting to cellulose is limited by the nature of the functional groups, degradation of the support polymer under the reaction conditions, and economic aspects.[159] These factors account for the fact that methods for grafting cellulose have not been introduced into industry.

The mechanical properties of polyacrylamide-grafted and non-grafted cellulose fibres have been investigated by means of a torsional pendulum technique.[160] The influence of temperature and humidity on the logarithmic decrement and the torsional rigidity were followed. At the temperature at which the synthetic polymer softened, the grafted fibre exhibited its maximum value in the logarithmic decrement. Apparently grafting did not reduce the mobility of the synthetic polymer. Similar results were obtained with changes in humidity, the grafted fibre retaining its torsional rigidity up to the critical humidity value.

The viscosity behaviours of low-yield poly(acrylic acid)-, poly(acrylonitrile)-, and poly(ethyl acrylate)-grafted celluloses in cupriethylenediamine, cuprammonium hydroxide, and cadoxen [tris(ethylenediamine)cadmium dihydroxide] have been studied.[161] Only low-yield grafted celluloses dissolved completely. The intrinsic viscosities of solutions of the grafted celluloses were reduced as a result of contraction of the chain.

Graft polymerization of acrylonitrile and ethyl acrylate onto cellulose has been induced by quadrivalent cerium; vigorous agitation greatly decreased the amount of homopolymer formed, the main effect being on the propagation process.[162] The work suggested that growing polymer radicals are produced mainly through the transfer of radicals formed at the cellulose backbone to the monomer, where propagation takes place. Growing polymer radicals either recombined with active sites on the cellulose, leading to grafting, or coupled with each other and/or were oxidized with ceric ions to give the homopolymer.

Lactate dehydrogenase has been immobilized on carriers comprising cellulose grafted with methyl acrylate.[163] The supports are different from those usually employed, since a hydrophilic polymer is grafted with a hydrophobic one.

[157] M. A. Salam, M. Moshuzzaman, and M. Ahmed, *J. Indian Chem. Soc.*, 1974, **51**, 433.
[158] G. Gellf, D. Thomas, and G. Brown, *Biotechnol. and Bioeng.*, 1974, **16**, 315.
[159] C. Simionescu, in 'Macromolecular Chemistry—8 (Helsinki, 1972)', ed. K. Saarela, Butterworths, London, 1973, p. 335.
[160] A. de Ruvo and L. Brehde, *Cellulose Chem. Technol.*, 1973, **7**, 191.
[161] J. Schurz and O. Y. Mansour, *Cellulose Chem. Technol.*, 1973, **7**, 181.
[162] O. Y. Mansour and A. Nagaty, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1974, **12**, 141.
[163] M. Ghelardoni and V. Pestellini, *Ann. Chim. (Italy)*, 1973, **63**, 643.

Sulphuric acid has been found to induce a Trommsdorff effect in the radiation-induced grafting of styrene (in methanol) to cellulose.[164] The kinetics of the grafting of styrene to pre-irradiated cellulose acetate in the presence of methanol have been studied by labelling the active sites with bromine atoms.[165] The addition of methanol to styrene affected not only the grafting rates but also the molecular weights of graft chains. The integrated amounts of active sites participating in the grafting reactions were also affected by the constitution of monomer solutions, and varied roughly in proportion to the extent of swelling of the cellulose acetate. Grafting yields were highest throughout the duration of the grafting reactions for a styrene : methanol ratio of unity.

Modified viscose fibres have been obtained by grafting acrylonitrile onto cellulose, making use of a redox system (cellulose xanthogenate–hydrogen peroxide).[166] The effect of the extent of grafting on the structure of the fibres was studied by scanning microscopy; the results suggested that the physicomechanical properties of the fibres had not deteriorated. *X*-Ray examination of the fibres helped to define some of the structural changes that resulted from grafting.

Charonin Sulphate.—Charonin sulphate (a highly sulphated, cellulose-like glucan from a marine mollusc) showed a marked metachromatic effect on complexation with Methylene Blue, even at elevated temperatures.[28] The sign of the induced Cotton effect depended on the temperature at which the measurement was made.

Chitin.—An improved process for isolating chitohexasaccharide from acid hydrolysates of chitin has been described.[167]

Chitosan has been prepared by treating chitin with alkali under nitrogen; the effects of the reaction time and the temperature on the uptake of Cu^{II} ions by chitosan were examined.[138]

Chitin and chito-oligosaccharides can be de-*N*-acetylated by an enzyme (chitin deacetylase) from *Mucor rouxii*.[168] A glycol ether of chitin labelled with ^{14}C in the *N*-acetyl groups[168] and trimethylsilylated chitin[109] (see also p. 424) have been prepared.

Chitin has been used in the separation of glycoside hydrolases[169] and in the large-scale preparation of wheat-germ agglutinin[170] by affinity chromatography.

Cycloamyloses.—Cyclohexa-amylose hexahydrate crystallizes from water in the form of a water-inclusion complex.[171] Four of six water molecules are located outside the cyclohexa-amylose ring and form part of an extensive network of hydrogen bonds. The other two molecules of water are located within the aperture of cyclohexa-amylose near the molecular axis; these water molecules are hydrogen bonded to one another and one of them is also hydrogen bonded to two OH-6 groups. Potential-energy calculations indicated that the complex is of

[164] S. Dilli, J. L. Garnett, and D. H. Phuoc, *J. Polymer Sci., Part B, Polymer Letters*, 1973, **11**, 711.
[165] T. Yasukawa, Y. Sasaki, and K. Murakami, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1973, **11**, 2547.
[166] K. Dimov, P. Pavlov, and G. G. East, *Cellulose Chem. Technol.*, 1973, **7**, 451.
[167] S. K. Banerjee, I. Kregar, V. Turk, and J. A. Rupley, *J. Biol. Chem.*, 1973, **248**, 4786.
[168] Y. Araki and E. Ito, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 669.
[169] R. J. Sturgeon, *L'Actualité chimique*, 1974, **7**, 38.
[170] R. Bloch and M. M. Burger, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 13.
[171] P. C. Manor and W. Saenger, *J. Amer. Chem. Soc.*, 1974, **96**, 3630.

higher energy than are those with iodine, n-propanol, methanol, and potassium acetate.

C.d. studies of complexes of cyclohepta-amylose with dinucleotides have shown that the binding is sensitive to the stacking of the dinucleotides.[172] The change in the c.d. spectrum of each dinucleotide resulting from binding of cyclohepta-amylose was unique. Although earlier work suggested that cyclohepta-amylose might be used to probe the structures of nucleic acids, since binding to adenosine and inosine only need be considered, the present report has demonstrated that binding to other bases cannot be ignored.

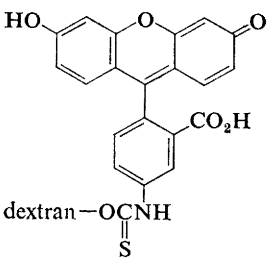
Dextran.—Isomalto-oligosaccharides have been reduced and tritiated with sodium borotritide, and the products were used to study the specificity of a dextranase.[173]

Soluble amino- and chloro-deoxydextrans have been obtained by way of nucleophilic-displacement reactions.[174]

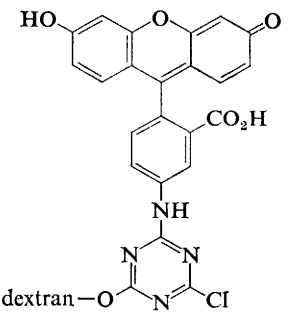
Blue Dextran has been used to determine the excluded volume of human-erythrocyte membranes,[175] and, following immobilization by reaction with the cyclic imidocarbonate derivative of macroporous agarose, in the isolation of lactate dehydrogenase by affinity chromatography.[65]

The interaction of uni- and bi-valent cations with carboxymethyldextran has been studied.[117]

Dextran reacted with isothiocyanatofluorescein (mixed 5- and 6-isomers) to give *O*-(fluoresceinylthiocarbamoyl)dextrans, *e.g.* (42).[176] Treatment with a



(42)



(43)

fluoresceinyltriazine derivative to give (43) was also successful. The degree of substitution (d.s. 0.01—0.001) of (42) was determined spectrophotometrically and the distribution of the fluorescein label was examined by gel chromatography.

The stabilities of dextran nitrates, prepared using either nitric acid or a mixture of acids, have been determined.[177] While the stabilities of the crude dextran nitrates varied according to the method of preparation, stabilized forms possessed

[172] C. Formoso, *Biopolymers*, 1974, **13**, 909.
[173] G. N. Richards and M. Streamer, *Carbohydrate Res.*, 1974, **32**, 251.
[174] V. V. Snegeko, K. P. Khomyakov, A. D. Virnik, and Z. A. Rogovin, *Izvest. Vysshikh Uchebn. Zavedenii Khim. i khim. Tekhnol.*, 1973, **16**, 309.
[175] C. A. Frey and W. P. Bryan, *Biochim. Biophys. Acta*, 1974, **356**, 156.
[176] A. N. de Belder and K. Granath, *Carbohydrate Res.*, 1973, **30**, 375.
[177] A. F. Dawoud and A. Marawan, *Stärke*, 1973, **25**, 273.

closely similar properties. Stabilization could be achieved by heating the material in water or in a dilute solution of sodium carbonate. Dextran nitrate is much less stable than cellulose nitrate.

Partly sulphated dextrans (d.s. 0.30—0.77) have been prepared by treating fractions of acid-degraded dextran either with chlorosulphonic acid in formamide or with the sulphur trioxide–pyridine complex in DMSO.[178] The locations of the sulphate groups were determined by methylation and by Smith degradation; the sulphate groups were distributed randomly, although O-2 appeared to be most readily sulphated. The results indicated that sulphation of dextran using the latter reagent gives a more homogeneous product than that obtained using chlorosulphonic acid.

Trimethylsilylated derivatives of dextran have been prepared by treating a solution of the polysaccharide in formamide with hexamethyldisilazane and precipitation of the silylated derivative with acetone.[109]

Dextran-coated charcoal has been used in the radioimmunoassay of luteinizing hormone, since it separated the free and the antibody-bound hormones,[179] and in similar radioimmunoassays of calcitonin and parathyroid hormone.[180] Comparisons have been made of the adsorption of thyroid-stimulating hormone and the hormone-antibody complex on to charcoal and a dextran-coated form thereof.[181] Coating with dextran appeared to offer no advantages, since it reduced the adsorption of both the complex and the free hormone to the matrix by reducing the number of free sites for adsorption.

The cyclic imidocarbonate derivative of Sephadex (a macroporous, cross-linked dextran) has been used to obtain active immobilized forms of D-amino-acid oxidase [30] and for the immobilization of trypsin and its derivatives (for use in a study of the denaturation of the enzyme).[38] A similarly immobilized form of rabbit immunoglobulin IgG anti-lysozyme has been used to isolate lysozymes by affinity chromatography.[47]

Carboxymethyl-Sephadex has been used to immobilize human serum albumin, chymotrypsinogen, RNA, nicotinamide adenine dinucleotide, glycyl-L-leucine, 6-aminopenicillanic acid, and L-phenylalanine methyl ester (see Scheme 8).[103]

Glycosaminoglycans.—*O*-β-D- Glucopyranuronosyl-(1 → 3)-[*O*-(2-acetamido-2-deoxy-D-galactopyranosyl 4-sulphate)-(1 → 4)-*O*-β-D-glucopyranuronosyl-(1 → 3)]$_2$-2-acetamido-2-deoxy-D-galactose 4-sulphate, a hexasaccharide derived from chondroitin 4-sulphate, has been modified by the enzymic addition of 2-[^{14}C]acetamido-2-deoxy-β-D-galactopyranose to the terminal, non-reducing end.[182]

The susceptibilities of the chondroitin sulphates, dermatan sulphate, hyaluronic acid, and heparin to β-elimination have been discussed.[110]

Fluorescein-labelled glycosaminoglycans have been prepared by methods already in use with dextrans; the properties and applications of the derivatives were described.[176]

[178] H. Miyaji and A. Misaki, *J. Biochem. (Japan)*, 1973, **74**, 1131.
[179] T. Sand and P. A. Torjesen, *Acta Endocrinol.*, 1973, **73**, 444.
[180] J. F. Habener, G. P. Mayer, D. Powell, T. M. Murray, F. R. Singer, and J. T. Potts, *Clinica Chim. Acta*, 1973, **45**, 225.
[181] M. A. Binoux and W. D. Odell, *J. Clinical Endocrinol. Metabol.*, 1973, **36**, 303.
[182] J. N. Thompson, A. C. Stoolmiller, R. Matalon, and A. Dorfman, *Science*, 1973, **181**, 866.

The hydrolytic properties of acyclic and cyclic sulphoamino-compounds having a neighbouring hydroxy- or *O*-sulphate group have been examined in connection with the chemical properties of heparin.[183] An *O*-sulphate group in a vicinal position accelerated the hydrolysis of sulphoamino-groups more than did a hydroxy-group, and the hydrolysis of a sulphoamino-group attached to a cyclic compound was faster than that attached to an acyclic compound, with the substituent exerting a greater effect in the *trans*-position. The effect of a β-substituent was less than that of the vicinal substituent. The marked lability of the sulphoamino-group in 2-deoxy-2-sulphoamino-D-glucose was ascribed to the overlapping of effects due to the hydroxy-groups, the pyranose-ring structure, and the ring-oxygen atom. The possibility of $N \rightarrow O$ sulphate migration under the experimental conditions was discussed.

N-[^{3}H]Acetylheparin has been prepared by acetylation of de-*N*-sulphated heparin with [^{3}H]acetic anhydride.[168]

Keratan sulphate containing 2-acetamido-3,6-anhydro-2-deoxy-D-glucosyl residues has been obtained by the action of alkali on the parent polysaccharide.[184]

In a study of the Cotton effects induced in acidic polysaccharides by thiazine dyes, it was found that, despite the similarity in structure of hyaluronic acid and the chondroitin sulphates, the signs of the Cotton effects were opposite.[28] The effects of pH and temperature on the induced Cotton effects were examined.

The interaction between glycosaminoglycans and chloroquine has been suggested to be of importance in the accumulation of antirheumatic concentrations of chloroquine in connective tissues.[123] The interactions of chondroitin 4-sulphate and dermatan sulphate with cationic polypeptides in solution have been investigated.[185]

Laminarin.—Chemical-ionization mass spectra (with ammonia and isobutane as reagent gases) have been reported for the peracetates of laminari-tetraose and -pentaose; applications of the technique to structure determination were discussed.[27]

Laminarin modified at both chain-ends, by periodate oxidation followed by reduction (sodium borohydride), has been used as a substrate for studies of a glucanase.[186]

Mannan.—The peracetate of the mannotetraose (44) has been examined by chemical-ionization mass spectrometry.[27]

$$\alpha\text{-D-Man}p\text{-}(1 \rightarrow 2)\text{-}[\alpha\text{-D-Man}p\text{-}(1 \rightarrow 2)]_{2 \text{ or } 3}\text{-D-Man}p$$

(44)

In a discussion of polysaccharides as models for stereoregular polymers, mannan has been considered as a model for syndiotactic polymers.[111] A number of aspects of mannans, including the preparation of derivatives, were discussed.

Pachyman.—Linear laminaridextrins (DP's 2—5) have been recovered from acid hydrolysates of pachyman by chromatography on charcoal-Celite.[186] Pachyman

[183] Y. Inoue and K. Nagasawa, *Carbohydrate Res.*, 1973, **31**, 359.
[184] S. Hirano and K. Meyer, *Connective Tissue Res.*, 1973, **2**, 1.
[185] R. A. Gelman and J. Blackwell, *Biopolymers*, 1973, **12**, 1959.
[186] K. Doi, A. Doi, T. Ozaki, and T. Fukui, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1629.

modified at both chain-ends, by periodate oxidation followed by reduction (sodium borohydride), has been used as a substrate for studies of a glucanase.

The use of pachyman for the separation of glycoside hydrolases by affinity chromatography has been reviewed briefly.[169]

Pectic Acid and Pectin.—Oligomers have been produced by the continuous flow of a solution of poly(D-galacturonic acid) over an immobilized polygalacturonase; separation of the oligomers was then achieved by gel filtration.[37]

The cleavage of pectin and pectic acid by β-elimination reactions has been discussed.[110]

Saponification of 95% methanol-esterified pectin (DP 45—50) (prepared by heating natural pectin with acidified methanol) furnished a series of pectins having various degrees of esterification.[187] 95% Glycol-esterified pectin was also prepared.

Pectin reacted with hexamethyldisilazane in formamide to yield a trimethylsilylated derivative, which was precipitated from solution with acetone in a form uncontaminated with ammonium chloride.[109]

Pustulan.—Ester, ether, and mixed analogues of the glycoside glycyrrhezin have been synthesized by coupling gentio-oligosaccharides to the carboxy- and/or hydroxy-groups of the aglycone.[188] The solubilities in water and the partition (water–oil) coefficients of the analogues increased with increasing length of the oligosaccharide chain, and coupling with the hydroxy-group of the aglycone gave more effective solubilization.

Starch.—Periodate-oxidized starch has been used to produce an immobilized form of papain, the amino-groups of the protein reacting with the aldehydo-groups of the matrix.[189]

A commercially available, dyed starch (Cibachron Blue F36-A) has been used to determine the amylase activities of sera and urine.[190] Fluorescein-labelled and derivatized starches have been prepared by methods already in use with dextrans.[176] Uses of the modified starches were discussed.

The rates of hydrolysis of 2- and 6-*O*-hydroxyethylstarches with α-amylase decreased with increasing d.s. or molecular substitution.[191] 2-*O*-Hydroxyethyl-D-glucosyl residues appeared to be more resistant to the hydrolytic action of α-amylase than the 6-*O*-hydroxyethyl counterparts. In addition to D-glucose, hydrolysis of hydroxypropylstarch (d.s. 0.07) with acid liberated 2- and 6-*O*-[2(*RS*)-hydroxypropyl]-D-glucopyranoses, 1,2-*O*-[2,1(*R*)-propylene]- and 1,2-*O*-[2,1(*S*)-propylene]-α-D-glucopyranoses, and 1,2-*O*-[2,1(*R*)-propylene]-α-D-glucofuranose.[192] The data indicated that the hydroxypropylstarch contains over 80% of the ether groups at O-2 and about 7% at O-6 of the D-glucosyl residues.

The rate of oxidation of starch with chlorine in buffered systems was highest at pH 7, and the pH decreased when the oxidation was carried out in an unbuffered

[187] W. Pilnik, F. M. Rombouts, and A. G. J. Voragen, *Mikrobiol. Technol. Lebensmitt*, 1973, **2**, 122.
[188] K. Takiura, S. Honda, M. Yamamoto, H. Takai, M. Kii, and H. Yuki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1618.
[189] F. B. Weakley and C. L. Mehltretter, *Biotechnol. and Bioeng.*, 1973, **15**, 1189.
[190] A. Mazzuchin, C. Weggel, and C. J. Porter, *Clinical Chem.*, 1973, **19**, 1187.
[191] M. Yoshida, T. Yamashita, J. Matsuo, and T. Kishikawa, *Stärke*, 1973, **25**, 373.
[192] D. C. Leegwater, J. W. Marsman, and A. Mackor, *Stärke*, 1973, **25**, 142.

system.[193] The oxidation was slower when the hydroxy-groups at C-2 and C-3 were substituted. Comparison of the oxidations with chlorine in water and in deuterium oxide indicated that the formation of protons in acidic media and the repulsion between anions in basic media decrease the rate of oxidation.

Mixtures of starch and L-fucose and of starch and tri-*N*-acetylchitotriose have been cross-linked with epichlorohydrin; the products were used to purify the anti-H haemagglutinins of eel sera and of the seeds of *Cytisus sessilifolius*.[194]

Cross-linking of partially hydrolysed starch with butane-1,4-dioldiglycide produced a three-dimensional lattice that swelled in water.[195] The degree of swelling and susceptibility to α-amylase were governed by the number of cross-linkages formed. The cross-linked material reacted with Cibachron Blue to give a product that was used in assays of α-amylase.

Cross-linked, periodate-oxidized starch has been used for the immobilization of papain.[189]

Xanthates of cross-linked starches of low amylose content imparted better dry tensile properties to styrene–butadiene rubber than those derived from starches with high contents of amylose.[196]

Solutions of graft copolymers of starch and either poly(acrylic acid) or polyacrylamide had lower viscosities than solutions of native starch.[197] The coil-density of the dissolved molecules was increased by grafting, whereas the limiting viscosity number and the dependence of the viscosity on concentration were decreased. Higher concentrations of the graft copolymers were needed to give network solutions than were required with native starch. Mixtures of starch and either poly(acrylic acid) or polyacrylamide in solution had a somewhat lower viscosity than starch, and it was concluded that molecules of the synthetic polymer can penetrate into coils of the polysaccharide, resulting in contraction of the coils.

Acrylonitrile has been grafted onto both gelatinized and granular starches in aqueous media, using ceric ammonium nitrate as the initiator.[198] The molecular weights and dispersities of the polyacrylonitrile side-chains were determined (gel-permeation chromatography) for various ratios of the initiator and polysaccharide. Conversion–time plots for the acrylonitrile polymers in both systems showed characteristics that could be explained on the basis of buried polymer radicals. Graft copolymerization of acrylonitrile onto starch, at least during the initial stages, is not suitably explained by a kinetic scheme involving the termination of polymer radicals with Ce^{IV} ions.

Cerium(IV)-ion-initiated, graft polymerization of acrylonitrile onto gelatinized DEAE–starch gave stable, latex-like dispersions of the copolymer.[199] The physicochemical properties of the copolymer were examined.

[193] K. F. Patel, H. U. Mehta, and H. C. Scrivastava, *Stärke*, 1973, **25**, 266.
[194] I. Matsumoto and T. Osawa, *Biochemistry*, 1974, **13**, 582.
[195] W. C. Barnes and A. B. Blakeney, *Stärke*, 1974, **26**, 193.
[196] H. C. Katz, W. F. Kwolek, R. A. Buchanan, W. M. Doane, and C. R. Russell, *Stärke*, 1974, **26**, 201.
[197] E. Gruber, A. Rahman, and J. Schurz, *Stärke*, 1974, **26**, 237.
[198] L. A. Gugliemelli, C. L. Swanson, and W. M. Doane, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1973, **11**, 2451.
[199] L. A. Gugliemelli, C. L. Swanson, F. L. Baker, W. M. Doane, and C. R. Russell, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1974, **12**, 2683.

The use of starch as a matrix for the purification of glycoside hydrolases by affinity chromatography has been briefly reviewed.[169]

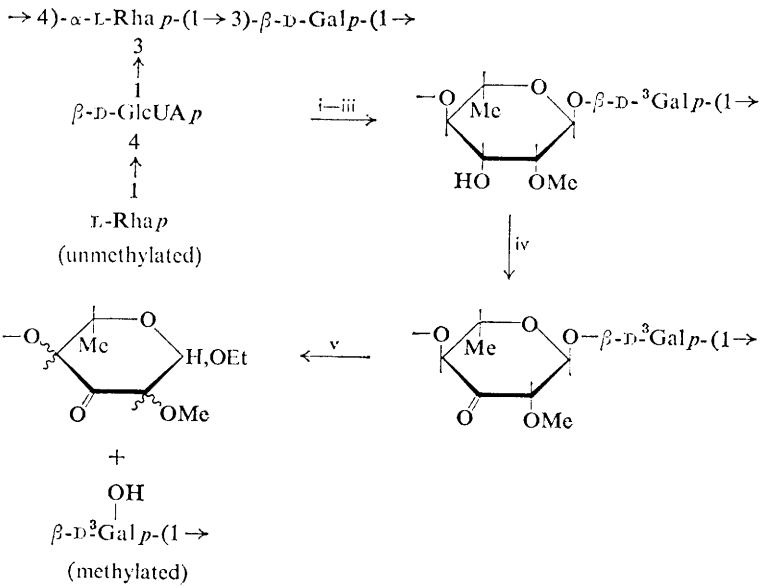
A process for the encapsulation of individual granules of potato starch with polyethylene has been described.[200] The process is based on the activation of vanadium trichloride, precipitated on the surface of the granules, in the presence of ethylene; encapsulation of individual granules was achieved when the polymerization of ethylene was conducted in a non-solvent. The growth of the encapsulation shell was studied as a function of the reaction time and temperature, the concentration of the vanadium catalyst, and the moisture content of the starch.

Xylan.—The conversion of xylan into the trimethylsilyl ether has been achieved by reaction with hexamethyldisilazane in formamide solution and subsequent precipitation to give a derivative uncontaminated with ammonium chloride.[109]

Miscellaneous.—A polysaccharide comprising 2-acetamido-2-deoxy-D-galactose from *Aspergillus parasiticus* has been deacetylated and afterwards acetylated with [^{3}H]acetic anhydride.[168]

A capsular polysaccharide from a *Klebsiella* species and a lipopolysaccharide from *Salmonella typhimurium* have been used to demonstrate a new, specific degradation of polysaccharides that affords information on the sequence of sugar residues (see Schemes 11 and 12).[25]

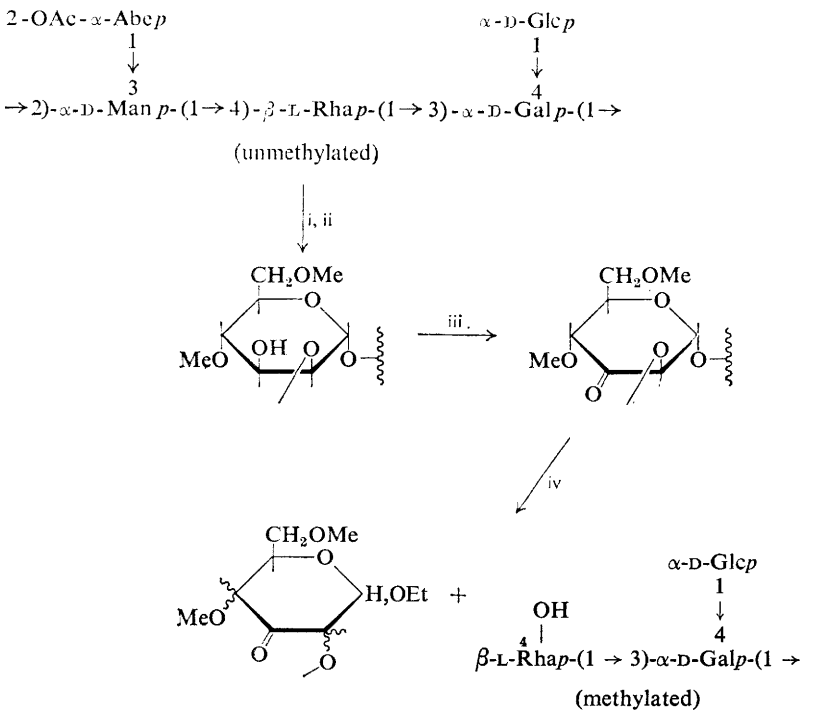
A peptidophosphogalactomannan (glycopeptide) from *Penicillium charlesii* and a peptidomannan produced by a mutant of the organism have been coupled to



Reagents: i, MeI–DMSO; ii, NaOEt; iii, H^+; iv, RuO_4; v, NaOEt–EtOH–CH_2Cl_2

Scheme 11

[200] H. D. Chanzy and J. F. Revol, *Stärke*, 1973, **25**, 131.



Reagents: i, MeI–DMSO; ii, H^+; iii, RuO_4; iv, NaOEt–EtOH–CH_2Cl_2

Scheme 12

bovine serum albumin following treatment with cyanogen bromide.[201] The products were used to investigate the binding of the glycopeptide and derivatives thereof to specific antibodies.

A succinoglucan from *Alcaligenes faecalis* has been desuccinylated with alkali.[202]

Chemical-ionization mass spectra, with either ammonia or isobutane as the reagent gas, have been reported for the peracetates of various oligosaccharides, including stachyose (45) and nystose (46); the technique complements electron-impact mass spectrometry in the structure determination of oligosaccharides.[27]

α-D-Gal*p*-(1 → 6)-α-D-Gal*p*-(1 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*

(45)

β-D-Fru*f*-(2 → 1)-β-D-Fru*f*-(2 → 1)-α-D-Glc*p*

(46)

Modification of Glycoproteins and Uses of Modified Glycoproteins

The modification of glycoproteins by attachment to polysaccharide-based matrices and the production of immunoadsorbents by analogous derivatization of immunoglobulins have been reviewed.[16]

[201] J. E. Gander and J. A. Rudbach, *Immunochemistry*, 1973, **10**, 81.
[202] A. Amemura, K. Moori, and T. Harada, *Biochim. Biophys. Acta*, 1974, **334**, 398.

D-Galactosyl and/or D-mannosyl residues in a glycopeptide from *Penicillium charlesii* formed cyclic imidocarbonates when treated with cyanogen bromide.[201] The derivatized form reacted with bovine serum albumin to give a product that was used to investigate the primary binding of specific antibodies.

Conditions necessary for the formation of complexes between concanavalin A and teichoic acids have been investigated, and a mechanism was suggested for complex formation.[203]

Graft copolymers of collagen and poly(methyl methacrylate) have been prepared in a number of methanol–water systems in the presence of neutral salts.[204] The compositions of the copolymers were determined by hydrolysis of the collagen backbone and measurement of the mol. wt. of the grafted branches. Sulphate and chloride anions were found to decrease the number of grafting sites on collagen more than did nitrate ions. However, the number of sites was virtually constant for a particular anion, irrespective of the proportion of methanol in the solvent, although the mol. wt. of the side-chains depended on the proportion of water present.

The removal of *N*-acetylneuraminic acid residues from particulate sialoglycoproteins, using an endogenous, membrane-bound neuraminidase present in calf brain, has been described.[205] Progressive desialylization of human chorionic gonadotrophin did not affect the immunological activity.[206] However, removal of 25% of the *N*-acetylneuraminic acid residues reduced the biological activity by 75%, and desialylization up to 62% reduced it further. Complete desialylization produced no further reduction of activity, the preparation obtained possessing a low, but significant, biological activity.

The biological activity of luteinizing hormone decreased abruptly on nitration or acetoacetylation or reduction and carboxymethylation.[207] The immunological properties of the hormone were destroyed by complete oxidation or reduction or carboxymethylation, but were retained after nitration or acetoacetylation or partial oxidation. The effects of these modifications on the molecular weight and electrophoretic mobility of the hormone were investigated.

Human serum albumin has been coupled to hydroxocobalamin by a carbodiimide-mediated reaction; the product was further coupled to bromoacetylcellulose for use in the purification of cobalamin-binding proteins.[144]

Bovine serum albumin and immunoglobulin modified by reaction with the lactosyl hapten (3) (see p. 405) have been used for the generation of anti-hapten antibodies.[7] Derivatives containing the tritiated hapten were also prepared. Bovine serum albumin has also been modified by reaction with 6-(4-diazobenzamido)nicotine; the distribution of nicotinyl groups along the macromolecular chain was determined from the stoicheiometry of the amino-acids liberated by hydrolysis and from the electrophoretic behaviour of the conjugate on polyacrylamide gel.[208]

[203] T. J. Kan, R. J. Doyle, and D. C. Birdsell, *Carbohydrate Res.*, 1973, **31**, 401.
[204] K. P. Rao, K. T. Joseph, and Y. Nayudamma, *Makromol. Chem.*, 1974, **175**, 729.
[205] A. Preti, A. Lombardo, G. Tettamanti, and V. Zambotti, *J. Neurochem.*, 1973, **21**, 1559.
[206] E. V. Van Hall, J. L. Vait Ukaitis, G. T. Ross, J. W. Hickman, and G. Ashwell, *Endocrinology*, 1971, **88**, 456.
[207] F. Y. Ryshka, N. K. Assonova, O. N. Savchenko, and G. S. Stepanov, *Biokhimiya*, 1974, **39**, 173.
[208] H. Matsushita, M. Noguchi, and E. Tamaki, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1006.

The binding of Butyl Orange by bovine serum albumin has been determined at various temperatures by equilibrium dialysis, and first-binding constants and thermodynamic parameters for formation of the dye–albumin complex were obtained.[209] The binding constant reached a maximum at 18 °C, and it was concluded that the reaction is exothermic above and endothermic below this temperature.

N-Acetylneuraminic acid residues of fetuin have been removed by hydrolysis in 0.03M-H_2SO_4 at 80 °C for 1 h, whereafter the partially hydrolysed material was labelled with ^{125}I.[210] The binding of this modified fetuin to isolated Golgi apparatus was similar to that to liver-plasma membranes. A tritium label has also been introduced into exposed, terminal, non-reducing D-galactosyl residues of desialylized fetuin.[104]

Neuraminidase removed *N*-acetylneuraminic acid from transferrins, which were then purified by ion-exchange chromatography.[211] The rates of catabolism of the modified transferrins in humans, rats, and rabbits were higher than those of the parent materials, and catabolism was markedly enhanced when desialylized human transferrin was injected as a heterologous macromolecule.

Electrochemical reduction of immunoglobulins has been described, and polarographic studies were reported for human immunoglobulin IgA.[212]

Treatment of porcine submaxillary mucin glycoprotein with 0.005M-H_2SO_4 at 70 °C for *ca.* 1 h removed the *N*-acetylneuraminic acid residues.[213]

The amino-groups of human type-K Bence-Jones glycoproteins have been derivatized with 2,4,6-trinitrobenzene 1-sulphonate, and the order of reactivity of the L-lysine residues in the variable, switch, and constant regions was established.[214]

The reaction of ovalbumin, L-ascorbic acid, and copper(II) sulphate has been examined at various pH values; non-protein nitrogen compounds were formed in greatest amounts at neutral pH.[215] The amount of bond cleavage occurring under various conditions was also investigated. Related reactions in which L-ascorbic acid was replaced with triose reductone were also studied.

The glycopeptide (47) afforded the reduced oligosaccharide (48) following treatment with an *endo*-β-D-acetamidodeoxyglucosidase and sodium borotritide.[216]

(D-Man*p*)$_6$-(D-Glc*N*Ac*p*)$_2$-Asn (47)

(D-Man*p*)$_6$-2-acetamido-2-deoxy-D-[^{3}H]glucitol (48)

Rabbit erythrocytes have been treated with neuraminidase with a view to assessing the role of the *N*-acetylneuraminic acid residues of glycoproteins in the survival of the erythrocytes in the circulation.[217]

[209] T. Takagishi, K. Takami, and N. Kuroki, *J. Polymer. Sci., Part A*-1, *Polymer Chem.*, 1974, **12**, 191.
[210] J. R. Riordan, L. Mitchell, and M. Slavik, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 1373.
[211] E. Regoeczi, M. W. C. Hatton, and K.-L. Wong, *Canad. J. Biochem.*, 1974, **52**, 155.
[212] M. Fontaine, C. Rivat, C. Ropartz, and C. Caullett, *Bull. Soc. chim. France*, 1974, 1513.
[213] N. Kochibe, *J. Biochem.* (*Japan*), 1973, **74**, 1141.
[214] T. Shinoda and Y. Tsuzukida, *J. Biochem.* (*Japan*), 1974, **75**, 23.
[215] S. Homma, K. Osawa, and C. Inagaki, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 2465.
[216] M. Nishigaki, T. Muramatsu, and A. Kobata, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 638.
[217] L. Gategno, D. Bladier, and P. Cornillot, *Carbohydrate Res.*, 1974, **34**, 361.

Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Tables of glycoproteins, immunoglobulins, *etc.*	Reaction with, or adsorption onto, various polysaccharide derivatives	Affinity chromatography and immunoadsorption	16
Albumin	Glutaraldehyde cross-linked with papain on iron oxide	Study of the use of enzymes immobilized on a magnetic support in a fluidized bed reactor	218
[^{125}I]-Albumin	Reaction with agarose cyclic imidocarbonate	Demonstration of a simple method for activating agarose with cyanogen bromide for affinity chromatography	79
Anti-α-L-arabinofuranosidase		Study of the specificity of anti-sera to α-L-arabinofuranosidase	40
Anti-collagen		Isolation of specific collagen peptides by immunoadsorption	42
Anti-β-D-glucuronidase		Purification of β-D-glucuronidase by affinity chromatography	45
Anti-(hen egg-white lysozyme)		Isolation of human lysozymes by affinity chromatography	47
	Reaction with Sephadex cyclic imidocarbonate	Isolation of human lysozymes by affinity chromatography	47
Anti-hepatitis antigen	Reaction with agarose cyclic imidocarbonate	Immunoadsorbent in process for removing hepatitis antigen from blood and plasma	46
Anti-(human arylsulphatase A)		Enzyme immunoassay of human arylsulphatase A; studies of normal and mutant enzymes; recognition of cases of metachromatic leukodystrophy	41
Anti-(human α-foetoprotein)	Reaction with cyclic imidocarbonate derivative of agarose + phloroglucinol cross-linked with epichlorohydrin	Isolation of human α-foetoprotein by immunoadsorption	43
Anti-(mouse interferon)	Reaction with agarose cyclic imidocarbonate	Purification of interferons by affinity chromatography	51
Anti-polygalacturonases		Study of the specificity of anti-sera to the polygalacturonases	40

Anti-(rabbit immunoglobulin) or Fab fragment	Glutaraldehyde cross-linked with peroxidase	Production of peroxidase-labelled immunoglobulin and Fab-fragment conjugates with enhanced intracellular penetration	214
Anti-(rat collagenase)	Adsorption on to polypropylene tubes	Solid-phase radioimmunoassay of collagenase	220
Anti-(rat α_1-foetoprotein)	Reaction with agarose cyclic imidocarbonate	Purification of rat α_1-foetoprotein by affinity chromatography	44
Bovine serum albumin	Glutaraldehyde cross-linked with concanavalin A	Isolation of right-side-out vesicles of purified plasma membranes from eukariotic cells	221
	Glutaraldehyde cross-linked with immunoglobulin IgG	Isolation of sheep anti-rabbit immunoglobulin and anti-rat immunoglobulin IgG by immunoadsorption	219
	Reaction with agarose cyclic imidocarbonate	Experimental control in a study of the bactericidal effectiveness of immobilized peroxidases	35
[^{125}I]-Bovine serum albumin		Investigations of the binding of proteinaceous macromolecules to the matrix and the distribution of the coupled macromolecule throughout the agarose bead	80
Bovine submaxillary mucin glycoprotein	Reaction with agarose cyclic imidocarbonate	Purification of haemagglutinin from *Limulis polyphemus* by affinity chromatography	78
Collagen		Purification of anti-collagen by immunoadsorption	42
Concanavalin A	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of human-serum macromolecules as standards for the assessment of crossed immunoelectrophoresis as an analytical method for predicting the results of affinity chromatography	66
		Interaction with phosphodiesterase I for the production of an immobilized enzyme for the hydrolysis of polynucleotides	36
		Isolation of dopamine β-mono-oxygenase by affinity chromatography	68
		Separation of human urinary erythropoietin from glycoproteins of similar size by affinity chromatography	69

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
		Separation of indole-3-acetic acid oxidase–peroxidase into glycoprotein and non-glycoprotein fractions by affinity chromatography	67
	Glutaraldehyde cross-linked with bovine serum albumin	Isolation of right-side-out vesicles of purified plasma membranes from eukariotic cells	221
	Unspecified agarose derivative	Affinity chromatography of human-liver β-D-galactosidases (see Chapter 6, p. 341)	104
		Separation of Herpes simplex virus-induced antigens by affinity chromatography	108
[^{125}I]-Concanavalin A	Glutaraldehyde cross-linked to coliphage K29	Illustration of affinity-density perturbation (a new fractionation principle) in a membrane separation	222
Erythrocytes	Formalinization	Purification of phytohaemoagglutinins from Jack bean, wheat-germ lipase, *Ulex europeus* seeds, and lima bean (*Carolina sieva*) by affinity chromatography	223
Goat immunoglobulin IgG anti-β-D-glucuronidase	Reaction with agarose cyclic imidocarbonate	Purification of β-D-glucuronidase by affinity chromatography	45
Human serum albumin	Reaction with derivative (29B, see Scheme 7b, p. 416) of agarose cyclic imidocarbonate		103 [a]
	Reaction with carboxymethyl-Sephadex (see Scheme 8, p. 419)	Investigation of the use of isocyanides for the immobilization of biological molecules	103
	Reaction (see Scheme 8, p. 419) with epichlorohydrin and desulphated, oxidized agarose (see p. 419)		103
Human serum albumin covalently complexed with hydroxycobalamin	Reaction with bromoacetylcellulose	Purification of human intrinsic factor by affinity chromatography	144
Immunoglobulin IgG	Glutaraldehyde cross-linked with bovine serum albumin	Isolation of sheep anti-rabbit immunoglobulin IgG by immunoadsorption	219

Lotus tetraglobinus L-fucose-binding protein	Unspecified agarose derivative	Affinity chromatography of human-liver β-D-galactosidases (see Chapter 6, p. 341)	104
Ovomucoid	Reaction with agarose cyclic imidocarbonate	Purification of acyltrypsins by affinity chromatography	76
Rabbit immunoglobulin anti-(rat collagenase)	Adsorption onto polypropylene tubes	Solid-phase radioimmunoassay of collagenase	220
Rabbit immunoglobulin IgG anti-(hen egg-white lysozyme)	Reaction with agarose cyclic imidocarbonate Reaction with Sephadex cyclic imidocarbonate	Isolation of human lysozymes by affinity chromatography	47
Soybean agglutinin	Glutaraldehyde cross-linked	Investigation of the agglutination of erythrocytes and lymphocytes	56 [b]
	Reaction with agarose cyclic imidocarbonate	Purification of a specific anti-soybean agglutinin antibody by affinity chromatography	52
Wheat-germ agglutinin	Unspecified agarose derivative	Affinity chromatography of human-liver β-D-galactosidases (see Chapter 6, p. 341)	104

[a] The agarose beads were cross-linked with epichlorohydrin before conversion into the cyclic imidocarbonate; [b] Water-soluble derivative.

Preparations of numerous immobilized, insolubilized, and coupled glycoproteins have been described; these derivatives, together with their applications, are summarized in Table 6.[218–223] Radiolabelled bovine serum albumin has been used in a study of the binding of proteinaceous molecules to agarose cyclic imidocarbonate; it was concluded that radioautography is far more sensitive than fluorescent labelling for locating a conjugated species within a matrix.[80]

Immobilized forms of concanavalin A have continued to be used in the separation and purification of glycoproteins by affinity chromatography;[66, 69, 108] a number of enzymes, including some glycoside hydrolases, have been purified in this way, indicating that they are glycoproteins rather than proteins.[67, 68, 104] Active immobilized derivatives of glycoproteinaceous enzymes may be formed by either adsorption onto or complexing with immobilized concanavalin A.[36] The bound glycoprotein can be eluted with methyl α-D-glucopyranoside.[69] [^{125}I]-Concanavalin has also been immobilized by cross-linking to coliphage K29 with glutaraldehyde.[222] The complex associated with membrane fragments carrying different numbers and distributions of receptors to give conjugates that were separated by ultracentrifugation. This process was named affinity-density perturbation.

Erythrocytes have been modified by formalinization for general use as affinity adsorbents for lectins.[223]

Modification of Enzymes and Uses of Modified Enzymes

L-Cysteinyl residues of the β-D-acetamidodeoxyglucosidase from human blood have been reduced and carboxymethylated in connection with structural studies of the enzyme.[224]

The two forms of β-D-galactosidase from human liver have been treated with neuraminidase to remove the *N*-acetylneuraminic acid residues.[225] Treatment of the α-D-galactosidase from human spleen with hexamethylene di-isocyanate (a bifunctional, cross-linking reagent) gave a derivative having higher thermostability and resistance to protease than that obtained with butyl isocyanate.[226]

Treatment of porcine pancreatic α-amylase with 5,5′-dithio-bis(2-nitrobenzoic acid), iodoacetamidonaphthol, and Hg^{II} ions furnished materials in which the enzymic activity was retained, implying that thiol groups are not required for the manifestation of activity.[227] Certain derivatives of the thiol groups could be formed only after the removal of Ca^{II} ions from the enzyme. The pH-activity profiles of the modified enzymes were similar to that of the free enzyme, and studies of the derivatives were used to assign functions to groups at

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[219] S. Avrameas and T. Ternynck, *Immunochemistry*, 1971, **8**, 1175.
[220] A. Z. Eisen, J. Nepute, G. P. Stricklin, E. A. Bauer, and J. J. Jeffrey, *Biochim. Biophys. Acta*, 1974, **350**, 442.
[221] A. Zachowski and A. Paraf, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 787.
[222] D. F. H. Wallach, B. Kranz, E. Ferber, and H. Fischer, *F.E.B.S. Letters*, 1972, **21**, 29.
[223] R. W. Reitherman, S. D. Rosen, and S. H. Barondes, *Nature*, 1974, **248**, 599.
[224] J. A. Verpoorte, *Biochemistry*, 1974, **13**, 793.
[225] A. G. W. Norden and J. S. O'Brien, *Arch. Biochem. Biophys.*, 1973, **159**, 383.
[226] P. D. Snyder, jun., F. Wold, R. W. Bernlohr, C. Dullum, R. J. Desnick, W. Krivit, and R. M. Condie, *Biochim. Biophys. Acta*, 1974, **350**, 432.
[227] M. L. Steer, N. Tal, and A. Levitzki, *Biochim. Biophys. Acta*, 1974, **334**, 389.

the active site of the enzyme. By contrast, another investigation of the derivatization of porcine pancreatic α-amylase with 5,5′-dithio-bis(2-nitrobenzoic acid) has claimed that the completely derivatized molecule is enzymically inactive, with some molecules present in the disulphide-linked form.[228]

Bovine testicular hyaluronidase has been maleylated; since derivatization did not reduce the molecular weight, it was concluded that the enzyme is not composed of subunits.[229]

The disulphide bonds of hen egg-white lysozyme have been reduced and alkylated to give a material that did not cross-react with the native form at humoral level.[230] Anti-lysozyme antisera, induced with the aid of Freund's complete adjuvant, reacted with the denatured enzyme, whereas antisera induced in the absence of the adjuvant did not. It was concluded that the antibodies arise in response to derivatized forms of the immunogen, which, in turn, arise during emulsification in the adjuvant.

Periodate oxidation of the carbohydrate moiety of D-glucose oxidase from *Aspergillus niger* did not induce any significant changes in the catalytic properties, conformation, or thermal stability.[231] However, periodate oxidation reduced the thermal stability of the enzyme in the presence of sodium dodecyl sulphate. It was concluded that the carbohydrate moiety contributes to the stability of the enzyme without affecting the protein structure.

Incubation of porcine-kidney aminopeptidase (microsomal) with sialidase, α-D-mannosidase, and β-D-acetamidodeoxyglucosidase removed all of the *N*-acetylneuraminic acid residues, more than 60% of the neutral carbohydrate residues, and 45% of the 2-acetamido-2-deoxy-D-glucosyl residues.[232] The degraded and native enzymes differed in solubility and electrophoretic mobility (disc-gel electrophoresis), but no differences in catalytic activity or substrate specificity were found. The results suggest that the carbohydrate moieties of aminopeptidase (microsomal) are not involved in enzymic activity.

The modification of enzymes to yield active, water-insoluble or immobilized derivatives has continued to receive a good deal of attention. The preparation, properties, and applications of immobilized enzymes have been reviewed in a form suitable for the non-specialist.[233] Other reviews on the preparation and properties [16, 20, 21, 234] and on kinetic and medicinal aspects [235–237] of immobilized enzymes have also appeared.

Numerous preparations of enzymes immobilized by coupling to insoluble supports and other macromolecules have been reported during the past year. The compositions and applications of these derivatives are summarized in

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Table 7.[238–267] Further details of immobilized glycoside and polysaccharide hydrolases worthy of note are mentioned below.

Cross-linking of β-D-fructofuranosidase with glutaraldehyde onto iron oxide gave a low activity yield owing to inactivation of the enzyme; however, the activity recovered was higher when coupling was carried out in the presence of sucrose.[252] β-D-Fructofuranosidase entrapped in fibres of cellulose acetate was found to be very stable under operating conditions; a half-life of 5300 d was calculated for some preparations, and the activity of a preparation used to hydrolyse sucrose was unchanged after 5 years. The specific activity of the entrapped enzyme was 15—65% of that of the free enzyme. Conditions for the continuous hydrolysis of sucrose were investigated, and potential industrial applications of the process were discussed. β-D-Fructofuranosidase, α- and β-amylases, glucoamylase, and D-glucose oxidase yielded spongy immobilized membranes when irradiated in the presence of acrylamide.[238] The entrapped β-D-fructofuranosidase possessed a broader pH optimum than the free enzyme and was active at higher temperatures.

α-D-Galactosidase attached to a nylon support promises to be of use in a continuous flow reactor.[253]

The smallest particles of the material obtained by cross-linking β-D-galactosidase with glutaraldehyde to aminoethylcellulose possessed the highest enzymic

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Table 7 *Modification of enzymes by coupling to insoluble matrices and other macromolecules*

Enzyme	*E.C. number*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
Tables of enzymes	—	Reaction with or adsorption onto various polysaccharide derivatives	Active immobilized enzymes	16
Acetyltrypsin	—	Reaction with Sephadex cyclic imidocarbonate	Active immobilized enzyme for studies of the denaturation of trypsin	38
D-Amino-acid oxidase	1.4.3.3	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Reaction with agarose cyclic imidocarbonate Reaction with Sephadex cyclic imidocarbonate	Active immobilized enzyme used for the determination of D-amino-acids and analysis of the optical purity of L-amino-acids	30
Aminoacylase	3.5.1.14	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
Aminopeptidase (cytosol)	3.2.11.1	Reaction with aminoalkyl derivative of glass	Active immobilized enzyme, stability and utility tests	239
α-Amylase	3.2.1.1	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme for fluidized bed reactors	240
		Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Reaction with agarose cyclic imidocarbonate Reaction with Sephadex cyclic imidocarbonate	Active immobilized enzyme; studies of variations of matrix size; comparison of the properties of immobilized and free enzymes	31
β-Amylase	3.2.1.2	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Reaction with copolymer of acrylamide and acrylic acid	Demonstration of the preparation and stability of active immobilized enzyme	241
			Mixed immobilized enzyme for the conversion of starch into maltose	242 [b]
Asparaginase	3.5.1.1	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme for fluidized bed reactors	240

Table 7 (*cont.*)

Enzyme	*E.C. number*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
		Encapsulation in semipermeable nylon microcapsules	Active immobilized enzyme for *in vivo* study of intraperitoneally injected enzyme	243
		Entrapment in polyacrylamide gel	Active immobilized enzyme for the correction of inborn errors of metabolism	244
Azoaldolase	—	Unspecified matrix	Active immobilized enzyme	245
Catalase	1.11.1.6	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme for fluidized bed reactors	240
		Reaction with silanized, nickel-impregnated silica-alumina supports	Active immobilized enzyme for film-differentiation studies of the immobilized enzyme in tubular flow reactors	246, 247
Chymotrypsin	3.4.21.1	Entrapment in polyacrylamide gels	Active immobilized enzymes; determination of the properties of the immobilized enzyme	248
		Glutaraldehyde cross-linked on alumina, carbon, nylon, and silica	Active immobilized enzymes; production of pellicular immobilized enzymes	249
		Impregnation on cellulose followed by cross-linking with glutaraldehyde	Active immobilized enzyme for kinetic studies of steady-state and transient conditions	158
		Reaction with Enzacryl AA pre-substituted with aldehyde or acetal groups according to Scheme 8 (p. 419)	Active immobilized enzyme; investigation of the use of isocyanide for the immobilization of biological molecules	103
		Reaction with macroporous cellulose *trans*-2,3-carbonate	Active immobilized enzyme with high activity against macromolecular substrates	250, 251
Dipeptidyl peptidase	3.4.14.1	Glutaraldehyde cross-linked on alumina, carbon, nylon, and silica	Active immobilized enzymes; production of pellicular immobilized enzymes	249
β-D-Fructo-furanosidase	3.2.1.26	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Glutaraldehyde cross-linked on an amino-alkylsilyl derivative of iron oxide	Active immobilized enzyme on magnetic matrix	252
		Entrapment in fibres of cellulose acetate	Active immobilized enzyme for the continuous hydrolysis of sucrose	135
α-D-Galactosidase	3.2.1.22	Cross-linked on nylon	Active immobilized enzyme for use in a continuous flow reactor	253

β-D-Galactosidase	3.2.1.23	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme for use in fluidized bed reactors	240
		Entrapment in polyacrylamide gels	Active immobilized enzymes; determination of the properties of the immobilized enzyme	248
		Glutaraldehyde cross-linked to alkylamine derivatives of zirconium-oxide-coated, porous glass particles and of porous particles of titanium oxide	Active immobilized enzyme for the hydrolysis of lactose	254
		Glutaraldehyde cross-linked to aminoethyl-cellulose	Active immobilized enzyme; investigations of the enzyme reaction and the stability and attrition of the support material	100
		Reaction with alkylamine derivatives of porous glass particles coated with zirconium oxide	Active immobilized enzyme for the hydrolysis of acid whey	255
		Reaction with diazo-derivative of porous glass particles	Active immobilized enzyme for the production of low-lactose dairy products	256
Glucoamylase	3.2.1.3	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme for use in fluidized bed reactors	240
		Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Reaction with an aminoaryl derivative of glass	Active immobilized enzyme; investigation of the effects of shearing on the covalent bond between the enzyme and the carrier	257
D-Glucose oxidase	1.1.3.4	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzyme	238
		Entrapment in a cross-linked gel of 2-hydroxyethyl methacrylate containing poly(vinylpyrrolidone)	Active immobilized enzyme; comparison of the properties of the immobilized and free enzymes	258
		Entrapment in polyacrylamide on nylon netting	Mixed, active immobilized enzyme; enzyme electrode for the measurement of D-glucose	259 [d]
		Reaction with diazo-derivative of glass	Active immobilized enzyme for the continuous measurement of dissolved oxygen	260
		Reaction with diazo-derivative of poly-(acrylic acid)	Mixed, active immobilized enzyme; enzyme electrode for the measurement of D-glucose	259 [d]

Table 7 (*cont.*)

Enzyme	*E.C. number*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
β-D-Glucosidase	3.2.1.21	Adsorption onto untreated glass	Active immobilized enzyme	261
		Reaction with titanium chelate of glass		
Glutamate dehydrogenase	1.4.1.2	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; studies of catalytic and structural aspects of the immobilized enzyme	32
Glycogen phosphorylase	2.4.1.1	Glutaraldehyde cross-linked to an aminoalkyl derivative of glass together with N^6-(6-aminohexyl)adenosine 5′-phosphate	Active immobilized enzyme; investigation of the immobilization in an active conformation on a matrix substituted with an analogue of adenosine phosphate	33
		Reaction with agarose cyclic imidocarbonate		
α-Lactalbumin	see 2.4.1.22	Reaction with agarose cyclic imidocarbonate	Comparison of two forms of lactose synthetase by affinity chromatography	73
Lactate dehydrogenase	1.1.1.27		Active immobilized enzyme for coupled lactate dehydrogenase-tryptophanase system used in the assay of L-tryptophan	34
		Reaction with azido-derivative of graft copolymer of methyl methacrylate and cellulose	Active immobilized enzyme	163
Lactoperoxidase	—	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for studies of the bactericidal effect of immobilized peroxidase	35
Lysozyme	3.2.1.17	*NN*′-Carbonyldi-imidazole cross-linked in anhydrous DMF	Active immobilized enzyme; immobilization of the enzyme in non-aqueous media	262
		Reaction with polystyrene containing imidazole functions	Active immobilized enzyme (solvent soluble)	263
Lysozyme ('loop'-region)	see 3.2.1.17	Reaction with poly(DL-alanine)	Active, immobilized, water-soluble derivative; investigation of the genetic control of immune response towards the loop region	264
'Malic enzyme'	—	Reaction with agarose cyclic imidocarbonate	Immobilized enzyme for purification of anti-'malic enzyme' by affinity chromatography	50
Papain	3.4.22.2	Glutaraldehyde cross-linked with albumin onto iron oxide	Active immobilized enzyme for fluidized bed reactor	218
		Reaction with periodate-oxidized starch	Active immobilized enzyme	189

Pepsin	3.4.23.1	Reaction with 1,6-diaminohexane derivative of a copolymer of ethylene and maleic anhydride	Active immobilized enzyme	265
Peroxidase	1.11.1.7	Entrapment in polyacrylamide on nylon netting	Mixed, active immobilized enzyme; enzyme electrode for the measurement of D-glucose	259 [e]
		Glutaraldehyde cross-linked with anti-rabbit immunoglobulin or Fab fragment	Production of peroxidase-labelled, water-soluble antibody and Fab conjugates with enhanced intracellular penetration	219
		Reaction with agarose cyclic imido-carbonate	Active immobilized enzyme for studies of the bactericidal effect of immobilized peroxidase	35
		Reaction with diazo-derivative of poly-(acrylic acid)	Mixed, active immobilized enzyme; enzyme electrode for the measurement of D-glucose	259 [e]
Phosphatase (acid)	3.1.3.2	Glutaraldehyde cross-linked on alumina, carbon, nylon, and silica	Active immobilized enzymes; production of pellicular immobilized enzymes	249
Phosphodiesterase I	3.1.4.1	Complexation with concanavalin A immobilized by reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for the hydrolysis of polynucleotides	36
endo-Polygalact-uronase	— [c]	Reaction with agarose cyclic imido-carbonate in presence of poly-(D-galacturonic acid)	Active immobilized enzyme for large-scale preparation of oligomers of D-galacturonic acid	37
Proteinases	—	Entrapment by irradiation of acrylamide and the enzyme in a frozen state	Active immobilized enzymes	238
Pullulanase	3.2.1.41	Reaction with a copolymer of acrylamide and acrylic acid	Mixed immobilized enzyme for the conversion of starch into maltose	242 [f]
Ribonuclease	3.1.4.22/23	Glutaraldehyde cross-linked to BioGel-P	Immobilized enzyme for the purification of ribonuclease inhibitor by affinity chromatography	266
Trypsin	3.4.21.4	Adsorption onto stainless steel coated with titanium oxide	Active immobilized enzyme used in fluidized bed reactors	240
		Coreticulation with a copolymer of maleic anhydride and vinyl methyl ester on the surfaces of glass beads	Active immobilized enzyme; production of pellicular immobilized enzyme	249
		Glutaraldehyde cross-linked on an amino-alkylsilyl derivative of iron oxide	Active immobilized enzyme on a magnetic support	252
		Glutaraldehyde cross-linked to 1,6-diamino-hexane derivative of a copolymer of ethylene and maleic anhydride	Active immobilized enzyme	265

Table 7 (*cont.*)

Enzyme	*E.C. number*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
		Reaction with agarose and Sephadex cyclic imidocarbonates	Active immobilized enzyme for studies of the denaturation of trypsin	38
		Reaction with azido-derivative of agarose cyclic imidocarbonate (12) (see Scheme 7a, p. 415)	Active immobilized enzyme	39
		Reaction with azido-derivative of a silyl derivative of glass with linkage extension		
Tryptophanase	4.1.99.1	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme used in the assay of L-tryptophan	34
Urease	3.5.1.5	*NN'*-Carbonyldi-imidazole cross-linked in anhydrous DMF	Active immobilized enzyme; immobilization of the enzyme in non-aqueous media	262
		Entrapment in polyacrylamide	Active immobilized enzyme; enzyme electrode used in the determination of arginase	267

[a] Unless stated otherwise, the product is insoluble in water; [b] Co-coupled with pullulanase; [c] See chapter 6, p. 377; [d] Co-coupled with peroxidase; Co-coupled with D-glucose oxidase; [f] Co-coupled with β-amylase.

activity.[141] The total enzymic activity increased when the immobilized enzyme was subjected to a shear stress that decreased the average size of the particles. Thus, the loss of small particles from an enzyme reactor, although constituting a small weight loss, will result in a disproportionately large loss of activity; the loss of small particles is augmented by attrition as the reactor is stirred. The enzymic activity of β-D-galactosidase immobilized in polyacrylamide gels was found to pass through a maximum as the concentration of acrylamide used for polymerization was increased.[248] The supported enzyme was less stable than the free enzyme. β-D-Galactosidase has been attached to stainless steel and other dense carriers after activation of the surface with titanium oxide.[240] Thermal activation of the support before contact with the enzyme appeared to improve the catalytic activity and the stability of the immobilized enzyme, for which the optimum temperature was raised from 60 to 70 °C. This procedure was also used to immobilize α-amylase and glucoamylase, *etc.* The inactivated or spent catalyst could be regenerated by heating to 540 °C, re-coating, and then reapplying the enzyme. The hydrolysis of lactose in milk whey using immobilized β-D-galactosidases has been investigated. Coupling of the enzyme to a diazo-derivative of porous glass gave a re-usable catalyst suitable for the production of dairy products of low lactose content; the characteristics of β-D-galactosidases in immobilized form were investigated.[256] A comparison of β-D-galactosidases attached to alkylamine derivatives of zirconium oxide-coated particles of porous glass and to porous titanium oxide (by means of cross-linking with glutaraldehyde) showed that the stabilities of the materials depend on the source of the enzyme.[254] The kinetics, thermal profile, and operational half-life of a β-D-galactosidase immobilized on porous glass by the zirconium oxide process have been studied in detail, and it was suggested that the hydrolysis products of lactose or whey might be used to sweeten a number of dairy products.[255]

It has been shown that enzymes (*e.g.* β-D-glucosidase) can become attached to glass surfaces that have not been pretreated in some way, so that careful attention must always be given to the retention of an active enzyme by a vessel, even when new hard glass is used without any treatment other than washing.[261]

Comparisons of the properties of an α-amylase immobilized by reaction with the cyclic imidocarbonate derivatives of macroporous agaroses and Sephadexes revealed that the form immobilized with Sepharose 2B possesses the highest activity towards soluble starch, whereas that with Sepharose 6B possesses the highest activity towards phenyl α-maltoside.[31] Staining indicated that there are two forms of immobilized enzyme, *viz.* surface-bound and entrapped forms. Other data indicated that there is an optimal size of a matrix for stabilizing the enzyme. Immobilized α-amylase was inactivated by H_4edta, but, in the case of the entrapped form of the enzyme, the activity was partly restored in the presence of Ca^{II} ions.

Conditions have been sought for the immobilization of β-amylase on an acrylic copolymer that give maximum operational stability; an improvement was obtained by coupling the enzyme in the presence of either glutathione or bovine serum albumin.[241] β-Amylase and pullulanase were jointly coupled to the same matrix under similar conditions to give a material suitable for the conversion of starch into maltose in a packed-bed column.[242]

Since loss of activity of an immobilized enzyme during operation of a column reactor can occur in several ways, the decay of the activity of glucoamylase coupled to an arylamine derivative of porous glass has been examined to determine whether there is any rupture of bonds between the support and the enzyme.[257] It was shown that the bond energies are sufficient to prevent shearing of the enzyme under stresses that normally occur in chromatographic columns and packed-bed reactors during continuous operation. The effect of siloxane linkages on the stability of the composite was also discussed.

Lysozyme has been coupled to a polystyrene matrix containing imidazole residues in order to solubilize the complex in non-aqueous media.[263] Immobilization in this way shifted the pH optimum of the enzyme to a higher value. Lysozyme 'loop'-region attached to poly(DL-alanine) has been used to show that the production of antibodies to the 'loop' is genetically controlled by a unigenic dominant trait that is not linked to the major histocompatibility locus.[264] Comparisons were made of the immunological responses to conjugates of natural and synthetic 'loops'.

Polygalacturonase has been immobilized by reaction with a cyclic imidocarbonate derivative of agarose in the presence of poly(D-galacturonic acid) (*i.e.* a substrate for the enzyme); the immobilized enzyme was used to produce oligosaccharides from poly(D-galacturonic acid) in a continuous-flow system.[37]

Immobilized forms of D-glucose oxidase have continued to be used as enzyme electrodes; various technological developments have been necessary in order to present the enzyme in the correct location with respect to other reagents employed.[259, 260]

Modification of Gangliosides and Glycolipids, and Uses of Modified Gangliosides and Glycolipids

Ganglioside GM_1 has been tritiated specifically in the terminal, non-reducing D-galactosyl residue by oxidation with D-galactose oxidase, followed by reduction with sodium borotritide.[268] Use of the modified ganglioside as a substrate for studies of the isoenzymes of β-D-galactosidase in human liver revealed that it acted as a modifier for one only of the two isoenzymes. Ganglioside GM_1 and (49) and (50) (also labelled with tritium) have been used as substrates in investigations of the glycosphingolipid β-D-galactosidases in rat brain.[269]

β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
(49)

β-D-Gal*p*-(1 → 3)-β-D-Gal*NAc*p-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
(50)

Appropriate transferases of human origin have been used to convert the glycolipid 'H_1' (51) into 'A^a' (52) and 'B-1' (53) forms.[270] Ozonolysis of tritium-labelled[268] and acetylated glycolipids transformed the olefinic linkages into carboxy-groups, which were treated in turn with *N*-hydroxysuccinimide (in the

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[270] K. Stellner, S. Hakomori, and G. A. Warner, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 439.

presence of DCC) and an alkylamine derivative of glass (particles or plates).[271] The immobilized glycolipids were used to study the mechanism of synthesis of cell-contact-dependent glycolipids.

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*NAcp*-
(1 → 3)-β-D-Gal-(1 → 4)-D-Glc-(1 → 1)-ceramide

(51)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*NAcp*-
3 (1 → 3)-β-D-Gal-(1 → 4)-D-Glc-(1 → 1)-ceramide
↑
1
R

(52) R = α-D-Gal*NAcp*

(53) R = α-D-Gal*p*

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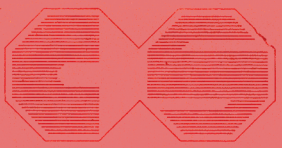

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