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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 7

A Review of the Literature Published during 1973

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Preface

This Report, the seventh in the series, covers the literature available to us between mid-January 1973 and mid-January 1974. For this and subsequent Reports, the usual format for Part II is modified slightly by making each of the chapters self-contained.

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.

Drs. N. A. Hughes and R. D. Marshall have joined our teams of Reporters for Parts I and II, respectively. We thank Professor N. K. Kochetkov for providing us once again with English abstracts of a large number of Russian papers, and Drs. L. C. N. Tucker and N. R. Williams for reading and commenting on the whole of Part I. Miss Moira Endersby typed considerable proportions of this Report.

This is the last Report for which we will have the benefit of Professor R. D. Guthrie's expert reporting, so severing his long association with this series both as its first Senior Reporter and latterly as a Reporter. His unstinted efforts over the past seven years have contributed very significantly to the success of these Reports.

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

July 1974 J. S. B.

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Abbreviations

The following abbreviations have been used:

ADP	adenosine diphosphate
ATP	adenosine triphosphate
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DBU	1,5-diazabicyclo[5,4,0]undec-5-ene
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dpm	dipivaloylmethane
e.s.r.	electron spin resonance
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphortriamide
i.r.	infrared
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
RNA	ribonucleic acid
THF	tetrahydrofuran
t.l.c.	thin-layer chromatography
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

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

By

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

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.

The synthesis of α-glycopyranosides has continued to attract a great deal of attention, no doubt prompted by Umezawa's contention (*Bull. Chem. Soc. Japan*, 1969, **42**, 529) that 'the preparation of α-glycopyranosides in high yields still remains the most important problem of carbohydrate chemistry'. Lemieux's group has described (Chapter 3) their approach to this problem, *via* the nitrosyl chloride–glycal procedure, in an eagerly awaited series of papers. Other varied and equally novel approaches to the synthesis of α-glycopyranosides are also covered in Chapter 3. A timely article by Schuerch [1] entitled 'Systematic Approaches to the Chemical Synthesis of Polysaccharides' has summarized the problems encountered in the stepwise synthesis of complex oligo- and poly-saccharides of known anomeric configuration.

The considerable interest in nucleosides has been sustained, with reports divided between synthetic (Chapter 21) and conformational (Chapter 23) aspects. The formation of halogenated sugar moieties on treatment of nucleosides with 2-acetoxyisobutyryl chloride (bromide) has opened up a very promising route to deoxy, epoxy, and unsaturated derivatives thereof (Chapters 7 and 21). Antibiotics containing rare sugars have received their customary attention (Chapter 20), and a number of exceedingly complex structures have been elucidated (*e.g.* everheptoses A and B, and the megalomicins) and, in some cases, synthesized (*e.g.* showdomycin).

Recent progress in the application of physical methods to the study of carbohydrates is dealt with in Chapters 23—26. In particular, Hall's group has demonstrated the potential value of longitudinal nuclear relaxation times as probes for structural assignments of carbohydrates. Geminal ^{13}C–^{1}H couplings at C-1 also appear to offer useful information on the anomeric configuration of monosaccharides. The application of *X*-ray crystallography to carbohydrate chemistry has shown a predictable increase. Free-energy calculations by Rao for the aldohexopyranose penta-acetates have suggested a smaller value (0.9 kcal mol^{-1}) for the anomeric effect of the acetoxy-group than hitherto assumed. A recent explanation of the

[1] C. Schuerch, *Accounts Chem. Res.*, 1973, **6**, 184.

anomeric effect in monosaccharide derivatives has revived and up-dated an earlier concept in which non-bonding electrons on the ring-oxygen atom are delocalized by mixing of a *p*-orbital with an antibonding σ-orbital of the C-1–X bond ('superjacent orbital control') in the α-anomer.[2]

A new type of literature coverage in the carbohydrate field has appeared with the publication of the volume on 'Carbohydrates' in the first series of biennial reviews in the MTP International Review of Science.[3] This publication aims to provide critical and well-documented articles covering actively developing areas of carbohydrate chemistry.

A brief obituary in Volume 31 of *Carbohydrate Research* has paid tribute to Dr. H. G. Fletcher, jun. (1917—1973).

The February[4] and June[4a] issues of *Carbohydrate Research* were dedicated to Professor V. Deulofeu and Dr. L. Long, jun., respectively, in celebration of their seventieth birthdays.

[2] S. David, O. Eisenstein, W. J. Hehre, L. Salem, and R. Hoffmann, *J. Amer. Chem. Soc.*, 1973, **95**, 3806.
[3] M.T.P. Internat. Review of Science, Series 1, Vol. 7, 'Carbohydrates', ed. G. O. Aspinall, Butterworths, London, 1973.
[4] *Carbohydrate Res.*, 1973, vol. 26.
[4a] *Carbohydrate Res.*, 1973, vol. 28.

2
Free Sugars

Reviews have been published on the chemistry of formose,[5] and on the physical properties of aqueous solutions of sucrose, D-glucose, and D-fructose.[6]

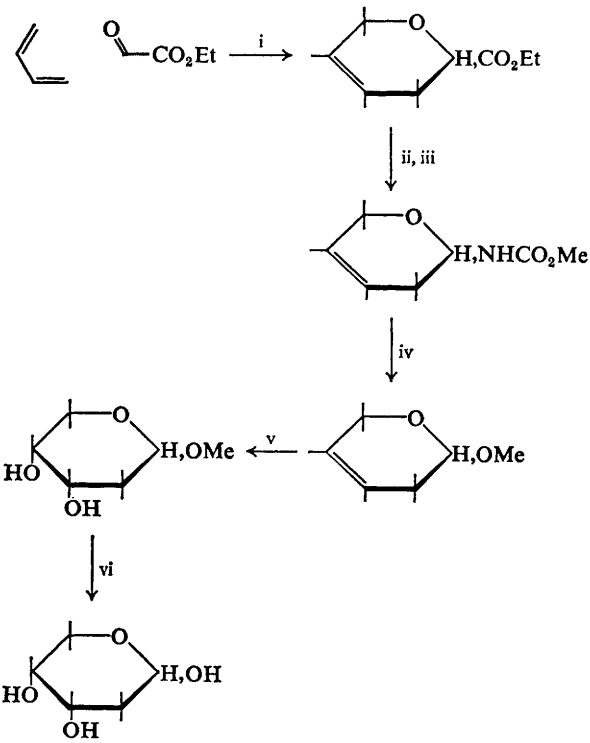
Isolation and Synthesis

Two reports [7, 8] have appeared describing the free sugar, cyclitol, and alditol contents of cannabis from various sources; the components identified included arabinose, D-*manno*-heptulose, *altro*-heptulose (sedoheptulose), D-*glycero*-D-*manno*-octulose, *myo*-inositol, quebrachitol, glycerol, erythritol, arabinitol, and xylitol. A number of free sugars, alditols, and glycosyl-alditols isolated from *Sphacelia sorghi* honeydew have been identified.[9]

L-Threose has been synthesized from (+)-tartaric acid,[10] and D-erythrose has been obtained by the oxidation of D-fructose with silver carbonate on Celite.[11] A synthesis of 2-deoxy-DL-*erythro*-pentose from non-carbohydrate precursors is shown in Scheme 1.[12] A simple synthesis of L-gulose from D-mannose has been achieved, the key step being a displacement with sodium acetate on the dimethanesulphonate (1) (Scheme 2).[13] An improved synthesis of D-altrose (as methyl α-D-altropyranoside, see Chapter 5) has also been described.[14] Acetolysis of 2,3-*O*-isopropylidene-L-rhamnofuranose or its diacetate gave a mixture of acetates, which, after deacetylation, gave L-quinovose (55%) as well as L-rhamnose, but epimerization at C-2 did not occur in L-rhamnopyranose derivatives.[15]

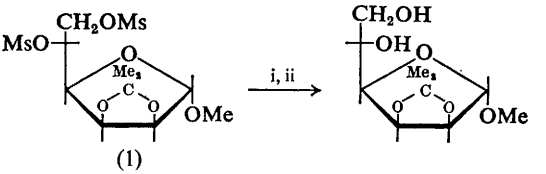
The first application of the Ivanov reaction in carbohydrate chemistry has resulted in a synthesis of the 1-deoxy-1-*C*-phenylketose (2) (Scheme

[5] T. Mizuno, *Kagaku No Ryoiki*, 1972, **26**, 762 (*Chem. Abs.*, 1972, **77**, 140 421*c*).
[6] R. S. Burdukova, M. N. Dadenkova, L. P. Zhmyrya, A. I. Orel, and B. S. Sluchanko, *Izvest. V.U.Z., Pishchevaya. Tekhnol.*, 1972, 37 (*Chem. Abs.*, 1973, **78**, 30 100*k*).
[7] G. Haustveit and J. K. Wold, *Carbohydrate Res.*, 1973, **29**, 325.
[8] J. W. Groce and L. A. Jones, *J. Agric. Food Chem.*, 1973, **21**, 211.
[9] R. L. Mower, G. R. Gray, and C. E. Ballou, *Carbohydrate Res.*, 1973, **27**, 119.
[10] G. Nakaminami, H. Edo, and M. Nakagawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 266.
[11] S. Morgenlie, *Acta Chem. Scand.*, 1973, **27**, 1557.
[12] V. B. Mochalin and A. N. Kornilov, *J. Gen. Chem.* (*U.S.S.R.*), 1973, **43**, 222.
[13] M. E. Evans and F. W. Parrish, *Carbohydrate Res.*, 1973, **28**, 359.
[14] M. E. Evans, *Carbohydrate Res.*, 1973, **30**, 215.
[15] P. J. Boon, A. W. Schwartz, and G. F. J. Chittenden, *Carbohydrate Res.*, 1973, **30**, 179.



Reagents: i, heat, 200 °C; ii, NH_3; iii, NaOCl–MeOH; iv, MeOH–BF_3–Et_2O; v, $KMnO_4$; vi, H_3O^+

Scheme 1



(1)

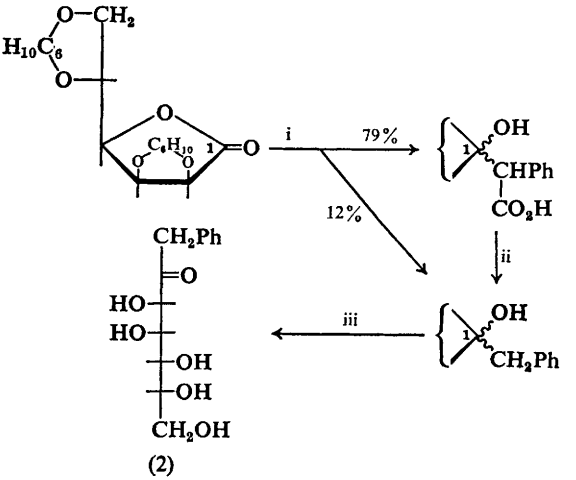
Reagents: i, NaOAc–DMF; ii, MeONa–MeOH

Scheme 2

3).[16] A synthesis of D-*manno*-3-heptulose (4) from a D-fructose derivative (3) is shown in Scheme 4; the unusual method of vicinal bishydroxylation is necessary since such conventional reagents as potassium permanganate or 3-chloroperbenzoic acid either failed or gave more-complex reaction products.[17] The related 2-deoxy-D-*arabino*-5-heptulose should be accessible by way of the product (5) of hydrogenolysis.

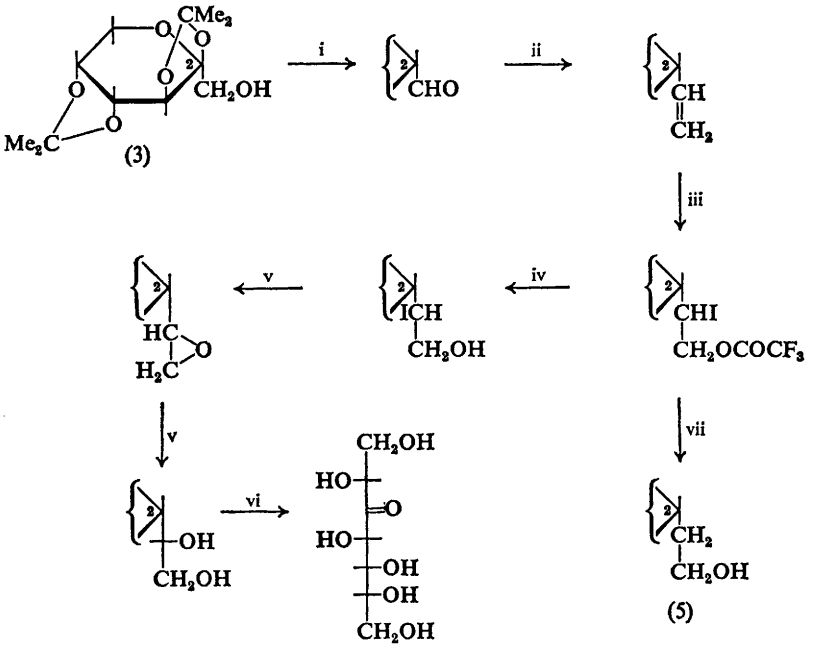
[16] Yu. A. Zhdanov, G. V. Bogdanova, and O. Y. Riabuchina, *Carbohydrate Res.*, 1973 **29**, 274.

[17] R. W. Lowe, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1973, **28**, 281.



Reagents: i, $PhCH_2CO_3H$–$Me_2CHMgCl$; ii, NaOH; iii, H^+

Scheme 3



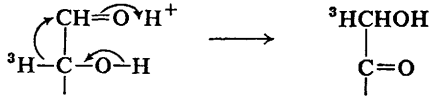
Reagents: i, DCC–DMSO; ii, $Ph_3P{=}CH_2$; iii, CF_3CO_2I; iv, MeOH–Et_3N; v, NaOH; vi, H^+; vii, H_2–Ni–Et_3N–MeOH

Scheme 4

Physical Measurements

A number of papers have described mutarotational studies. Polarography has been used in a kinetic study of the mutarotation of D-xylose.[18] The mutarotation of D-galactose and D-mannose has been examined in aqueous solution at 25 °C by calorimetric methods; β-D-galactopyranose is more stable than the α-anomer by 1300 ± 50 J mol^{-1}, and for D-mannopyranose, the α-anomer is more stable than the β-form by 1900 ± 80 J mol^{-1}.[19] The mutarotation of D-glucose in DMF at 70 °C was completed in a few hours, when three components (4.7, 42.5, and 52.8%) were present. Mass spectrometry of the trimethylsilylated derivatives showed the major products to be pyranoses and the minor product(s) to be a furanose or a mixture of furanoses.[20] From studies of the mutarotation of D-glucose in DMSO, it was proposed that the proton-catalysed reaction occurs in stepwise fashion, whereas the solvent-catalysed reaction may involve a concerted process.[21] A series of oxy-acids has been examined as catalysts for the mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose; thermodynamic data were reported for catalysis by diphenyl hydrogen phosphate, benzenephosphinic acid, trichloroacetic acid, benzoic acid, 2-pyridone, 2-aminopyridine, and picric acid in benzene solution.[22]

The kinetics of the base-catalysed transformations of D-glucose, D-mannose, and D-fructose have been interpreted[23] in terms of anionic intermediates, rather than an S_N2 pathway as recently proposed (E. R. Garrett and J. F. Young, *J. Org. Chem.*, 1970, **35**, 3502). In the acid-catalysed transformation of D-glucose into D-fructose, an intramolecular hydrogen-transfer from C-2 to C-1 was demonstrated by tritium-labelling studies (Scheme 5).[24] The pseudo-equilibria between D-glucose, D-mannose, and



Scheme 5

D-fructose were displaced in favour of D-fructose in the presence of equimolar proportions of areneboronic acids.[24a]

E.s.r. studies have been performed on radicals formed by irradiation of solutions of glycolaldehyde and glyceraldehyde in aqueous acetone,[25]

[18] T. Ikeda and M. Senda, *Bull. Chem. Soc. Japan*, 1973, **46**, 1650.
[19] K. Takahashi and S. Ono, *J. Biochem. (Japan)*, 1973, **73**, 763.
[20] J. A. Hveding, O. Kjolberg, and A. Reine, *Acta Chem. Scand.*, 1973, **27**, 1427.
[21] N. M. Ballash and E. B. Robertson, *Canad. J. Chem.*, 1973, **51**, 556.
[22] P. R. Roney and R. O. Neff, *J. Amer. Chem. Soc.*, 1973, **95**, 2896.
[23] Y. Z. Lai, *Carbohydrate Res.*, 1973, **28**, 154.
[24] D. W. Harris and M. S. Feather, *Carbohydrate Res.*, 1973, **30**, 359.
[24a] S. A. Barker, B. W. Hatt, and P. J. Somers, *Carbohydrate Res.*, 1973, **26**, 41.
[25] S. Steenken and D. Schulte-Frohlinde, *Tetrahedron Letters*, 1973, 655.

and also on D-glucose, 2-deoxy-D-*erythro*-pentose, and 2-deoxy-D-*arabino*-hexose.[26]

The effect of reducing and non-reducing sugars on the conductance of electrolyte solutions has been examined.[27] Thermal transformations and rearrangements of β-cellobiose and αα-trehalose have been investigated by a number of physical methods.[28] Anomerization, dehydration, condensation, and polymerization were all observed, and polymers formed contained both furanoid and pyranoid rings and unsaturated components. A kinetic study of the thermal decomposition of D-glucose and D-fructose at 300 °C has been reported.[29]

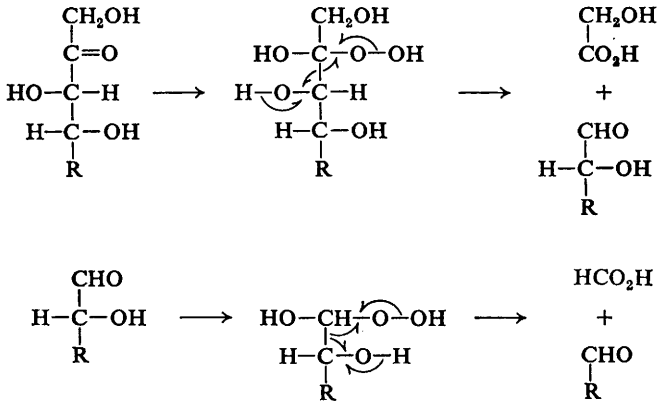
A model for the hydration of monosaccharides has been established on the basis of ^{17}O n.m.r. and dielectric-relaxation measurements; the model was used to explain the dependence of the conformational equilibrium of D-ribose on temperature.[30] SCF–MO calculations have been made on the electronic distribution in α- and β-D-glucopyranoses, β-D-arabinopyranose, 2-deoxy-β-D-*erythro*-pentopyranose, and the enediol form of D-*erythro*-pentulose.[31] In both anomers of D-glucopyranose, the charge on O-1 was calculated to be greater than that on the ring-oxygen atom, in keeping with its preferential protonation in acid solution, but the difference is greater for the β- form. The role of the enediol form of D-*erythro*-pentulose in the fixation of carbon dioxide was discussed.

Reactions

Alkaline solutions of hydrogen peroxide have been found to degrade both aldoses[32] and ketoses;[33] aldohexoses and aldopentoses give six and five moles of formic acid, respectively, whereas ketohexoses give one mole of glycolic acid and four moles of formic acid. The reaction probably involves initial addition of a hydroperoxide anion at the reducing centre, followed by fragmentation and subsequent repetition of this sequence (Scheme 6), the final carbon-fragment appearing as formaldehyde, which is further oxidized to formic acid. Experiments at different pH values suggested a free-radical mechanism, rather than an ionic mechanism, and this is supported by the observation that iron salts accelerate the reactions.

Up to 51% incorporation of non-labile tritium occurred on tritium-atom bombardment of crystalline D-glucose.[34] The distribution of the tritium

[26] V. A. Sharpatyi and M. N. Sultankhodzhaeva, *Doklady Akad. Nauk S.S.S.R.*, 1973, **208**, 1157 (*Chem. Abs.*, 1973, **78**, 148 145*g*).
[27] S. P. Moulik and A. K. Mitra, *Carbohydrate Res.*, 1973, **29**, 509.
[28] F. Shafizadeh and Y. Z. Lai, *Carbohydrate Res.*, 1973, **31**, 57.
[29] F. Orsi, *J. Thermal Analysis*, 1973, **5**, 329.
[30] M. J. Tait, A. Suggett, F. Franks, S. Ablett, and P. A. Quickenden, *J. Solution Chem.*, 1972, **1**, 131.
[31] Yu. A. Zhdanov, V. I. Minkin, R. M. Minjaev, I. I. Zacharov, and Yu. E. Alexeev, *Carbohydrate Res.*, 1973, **29**, 405.
[32] H. S. Isbell, H. L. Frush, and E. T. Martin, *Carbohydrate Res.*, 1973, **26**, 287.
[33] H. S. Isbell and H. L. Frush, *Carbohydrate Res.*, 1973, **28**, 295.
[34] T. C. Liang, P. Nordin, and H. C. Moser, *Carbohydrate Res.*, 1973, **27**, 437.



Scheme 6

was determined; none was detected at C-2, but twice the expected amount of tritium was found at C-5.

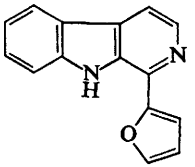
A number of deoxyhexuloses and deoxyhexodiuloses were formed by γ-irradiation of oxygen-free solutions of D-glucose and D-fructose.[35] γ-Irradiation of frozen, aqueous solutions of sugars resulted in epimerizations; for example, D-arabinose, L-lyxose, and D (?)-xylose were detected after irradiation of D-ribose.[36] D-Fructose and lactose, which are known to be particularly sensitive to degradation by γ-irradiation in solution, have been shown to be equally sensitive in the solid phase.[37]

The reactions of D-glucose and maltose with alkaline-earth hydroxides in the presence of ethylenediamine and 2-aminoethanol have been examined.[38] Epimerizations at C-2 and C-3 were observed on heating hexoses near to their melting points in the presence of such basic catalysts as calcium hydroxide or sodium carbonate.[39] A model reactor utilizing poly(4-vinylbenzeneboronic acid) resins for optimizing the formation of D-fructose from D-glucose has been described.[40] Transformations and degradations of sugars in acidic solutions have been studied.[41] Treatment of D-glucose with cold, concentrated sulphuric acid gave a polymer of sulphated D-glucose residues.[42] Isomerizations occurred when aqueous solutions of 4-*O*-methyl-D-glucuronic acid at pH 7 were heated to 100 °C, and the products observed were 3-*O*-methyl-D-*lyxo*-5-hexulosonic acid

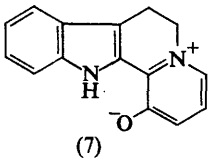
[35] S. Kawakishi, Y. Kito, and M. Namiki, *Carbohydrate Res.*, 1973, **30**, 220.
[36] N. K. Kochetkov, L. I. Kudrjashov, M. A. Chlenov, and T. Ya. Livertovskaya, *Carbohydrate Res.*, 1973, **28**, 86.
[37] T. Gejvall and G. Lofroth, *Acta Chem. Scand.*, 1973, **27**, 1108.
[38] S. P. Moulik and A. K. Mitra, *Carbohydrate Res.*, 1973, **28**, 371.
[39] F. Shafizadeh and Y. Z. Lai, *Carbohydrate Res.*, 1973, **26**, 83.
[40] S. A. Barker, B. W. Hatt, P. J. Somers, and R. R. Woodbury, *Carbohydrate Res.*, 1973, **26**, 55.
[41] K. Koizumi and K. Hashimoto, *Yakugaku Zasshi*, 1972, **92**, 1133 (*Chem. Abs.*, 1972, **77**, 152 469*n*).
[42] K. Nagasawa and Y. Inoue, *Carbohydrate Res.*, 1973, **28**, 103.

(47%), 3-*O*-methyl-L-*ribo*-5-hexulosonic acid (12%), 4-*O*-methyl-D-mannuronic acid (4%), and 3-*O*-methyl-L-*ribo*-4-hexulosonic acid (1%).[43]

Tryptophan and D-xylose reacted to give the heterocyclic compounds (6) and (7) when heated at 160 °C in neutral, aqueous solution.[44] In con-

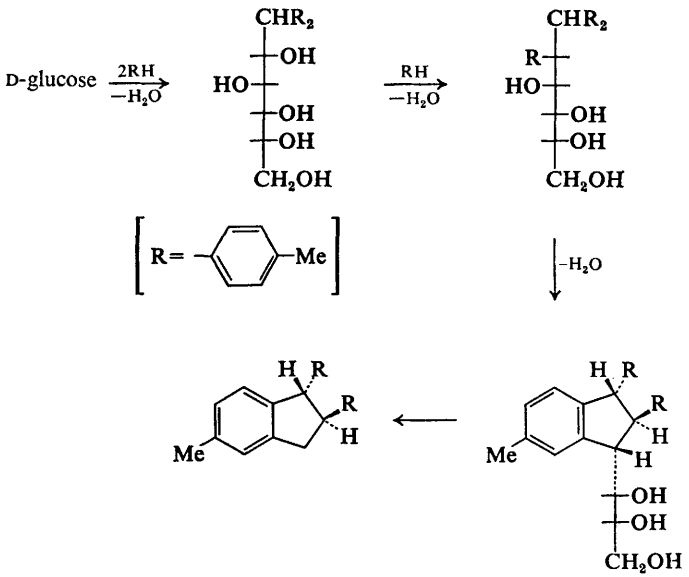


(6)



(7)

tinuing their work on the reactions of sugars with monocyclic aromatic systems in the presence of hydrogen fluoride, Micheel's group have described the properties of a number of their products.[45] One, a compound $C_{27}H_{24}$ from D-mannose and toluene, has the unusually high optical rotation of $[\alpha]_D^{20}$ −1264°. A second paper has reported the reaction of D-glucose with toluene to give optically active derivatives of hydrindane (see Scheme 7).[46]



Scheme 7

[43] K. Larsson and O. Samuelson, *Carbohydrate Res.*, 1973, **31**, 81.
[44] T. Severin and K.-H. Bräutigam, *Chem. Ber.*, 1973, **106**, 2943.
[45] F. Micheel, M. Pesenacker, H. Sobitzkat, E.-O. Killing, and G. Louis, *Carbohydrate Res.*, 1973, **26**, 278.
[46] F. Micheel and H. Sobitzkat, *Carbohydrate Res.*, 1973, **30**, 71.

An interesting investigation has been carried out on the structural requirements necessary for the binding of sugars to the sugar-transport system of human erythrocytes.[47] Studies with various D-glucose derivatives indicated that the sugar is bound as the β-pyranose form by means of hydrogen bonds at C-1 and C-3, and probably at C-4, and possibly at C-6. D-Glucal was shown to be a powerful inhibitor, indicating that a derivative with an appreciably distorted chair conformation can still bind to the sugar-transport system.

[47] J. E. G. Barnett, G. D. Holman, and K. A. Munday, *Biochem. J.*, 1973, **131**, 211.

3
Glycosides

O-Glycosides

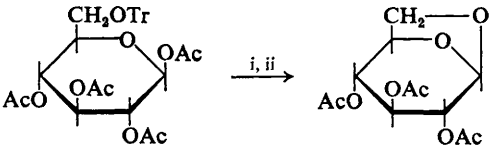
A review has appeared on the synthesis of linear polyglycosides (polysaccharides) with emphasis on the polymerization of 1,6-anhydrohexoses and cyclic 1,2-orthoesters,[48] and another review has dealt with the preparation of mono- and di-aminoglycosides of 2-deoxystreptamine.[49]

Synthesis.—Phenyl 1-thio-D-glucopyranosides have been shown to be readily solvolysed in the presence of mercury(II) salts to give, with good stereoselectivity, alkyl glycosides of inverted anomeric configuration. The method can be extended to the synthesis of complex glycosides if the hydroxy-groups of the glycosylating reagents are protected by benzylation; in particular, the procedure allowed the synthesis of α-D-glucopyranosides.[50] A further development in the synthesis of α-D-glucosides has utilized a double-displacement at the anomeric centre of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide.[51] Initial nucleophilic attack at C-1 was effected with either triethylamine or triphenylphosphine to give intermediate ammonium and phosphonium salts, which were then treated with methanol. The corresponding sulphonium salt, prepared using dimethyl sulphide, gave an 86% yield of the α-glycoside, and the reactivities of the intermediates to methanolysis were demonstrated to be in the order sulphonium > ammonium > phosphonium salts. The method has not yet been applied to the synthesis of complex α-D-glucopyranosides.

A novel modification to a standard synthesis of glycosides has used glycosyl acetates for the glycosylation of trityl ethers in the presence of allyl bromide and silver perchlorate.[52] It was suggested that the allyl cation formed from these reagents gives allyl acetate and the glycosyl carbonium ion, which is then attacked by the nucleophilic oxygen atom of the trityl ether. Applications of the method are illustrated in Scheme 8.

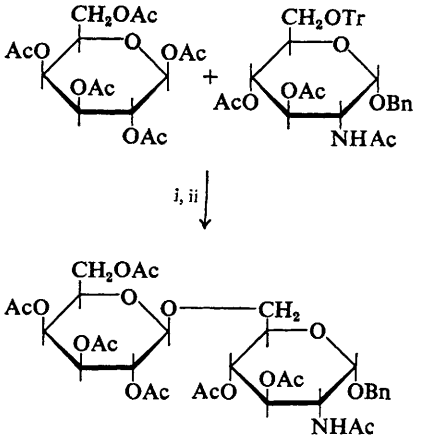
Another novel development followed from the observation that pyrolysis of the carbonate (8) gave the phenyl glycoside (9) as the main product,

[48] C. Schuerch, J. Zachoval, and B. Veruovic, *Chem. listy*, 1972, **66**, 1124 (*Chem. Abs.*, 1973, **78**, 16 361*q*).
[49] W. Meyer zu Reckendorf, *Deut. Apotheker-Z.*, 1972, **112**, 1617 (*Chem. Abs.*, 1973, **78**, 58 741*u*).
[50] R. J. Ferrier, R. W. Hay, and N. Vethaviyasar, *Carbohydrate Res.*, 1973, **27**, 55.
[51] A. C. West and C. Schuerch, *J. Amer. Chem. Soc.*, 1973, **95**, 1333.
[52] V. A. Nesmeyanov, S. E. Zurabyan, and A. Ya. Khorlin, *Tetrahedron Letters*, 1973, 3213.



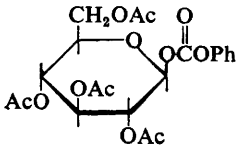
+ linear oligosaccharides (DP1—6)

Tr = CPh_3

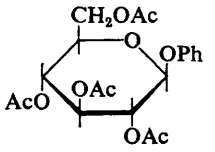


Reagents: i, $CH_2{=}CHCH_2Br$–$AgClO_4$; ii, Ac_2O

Scheme 8



(8)



(9)

together with diphenyl carbonate and the diglycosyl carbonate.[53] Fusion of the carbonate in the presence of a molar proportion of *p*-nitrophenol gave the *p*-nitrophenyl β-glycoside in good yield. Various purine β-glycosides were obtained similarly; thus, 2-hydroxypyridine and 2-hydroxy-4-methoxypyrimidine gave *O*-glycosylated compounds, which were converted into *N*-glycosylated isomers by treatment with mercury(II) bromide in xylene. Thus, a new route to nucleosides is provided (see also Chapter 21).

Methyl glycosides have continued to receive attention, and detailed studies of the methanolyses of D-fructose and L-sorbose (using ^{14}C-labelled sugars) have been reported.[54] As with aldoses, furanosides are the

[53] S. Inaba, M. Yamada, T. Yoshino, and Y. Ishido, *J. Amer. Chem. Soc.*, 1973, **95**, 2062.

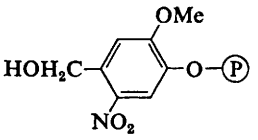
[54] G. S. Bethell and R. J. Ferrier, *Carbohydrate Res.*, 1973, **31**, 69.

main products of kinetic control, but pyranosides are present at equilibrium; no evidence was obtained for the presence of dimethyl acetals. The equilibrium percentages of glycosides (D-fructosides first) were as follows: α-pyranosides, 3, 92; β-pyranosides, 46, 1; α-furanosides, 25, 5; and β-furanosides, 26, 2%. Complete methylation of D-glucose and D-galactose with diazomethane in ether furnished high yields of the β-glycopyranoside tetramethyl ethers, in contrast with the anomeric mixtures of furanosides and pyranosides resulting from methylation of these sugars with methyl iodide and barium oxide.[55] The configuration of the free sugars at C-1 during alkylation with diazomethane is unknown, but the observed products may be derived by preferential reaction of anomeric, equatorial hydroxy-groups. Similar methylations of the penta-acetates of α- and β-D-gluco- and -galacto-pyranoses also gave the methyl β-glycopyranoside tetramethyl ethers selectively, but anomeric mixtures of the furanosides were produced in the cases of α- or β- D-galactofuranose penta-acetates. Treatment of free sugars with benzyl alcohol in benzene containing an acidic cation-exchange resin has afforded a means of obtaining benzyl glycosides; benzyl 2-deoxy-α-D-*arabino*-hexopyranoside, for example, was prepared by this method.[56]

The methanolysis of a number of derivatives of 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides has been studied both in the presence and in the absence of silver salts.[57] In the absence of these salts, the anomeric ratio of products was sensitive to the concentration of methanol, with high concentrations leading to β-glycosides and *vice versa*. In the presence of silver salts, mainly the β-D-glucoside was obtained, and these observations were rationalized and related to earlier reports.

Reference is made in Chapter 6 to solvolyses of glycosyl halides having *N*-phenylcarbamoyl and *N*-methyl-*N*-phenylcarbamoyl groups substituted at C-2.

Many applications and modifications of the Koenigs–Knorr reaction have continued to be reported. Another new application using solid supports has been developed by Zehavi and Patchornik.[58] 6-*O*-*p*-Nitrobenzoyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl bromide was used to glycosylate an insoluble resin containing the aromatic system (10). Removal of



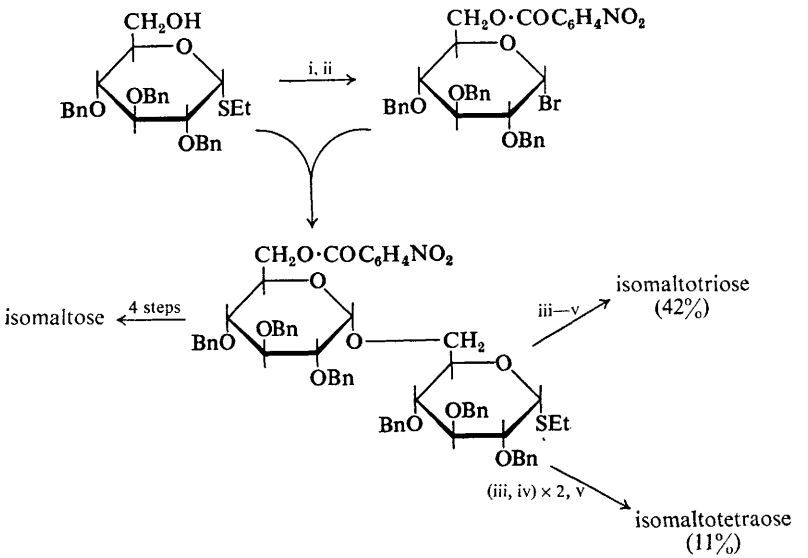
(10)

[55] M. E. Gelpi, J. O. Defarrari, and R. A. Cadenas, *Anales Asoc. quím. argentina*, 1973, **61**, 21 (*Chem. Abs.*, 1973, **78**, 160 008*x*).
[56] E. B. Sanders, *Carbohydrate Res.*, 1973, **30**, 190.
[57] F. J. Kronzer and C. Schuerch, *Carbohydrate Res.*, 1973, **27**, 379.
[58] U. Zehavi and A. Patchornik, *J. Amer. Chem. Soc.*, 1973, **95**, 5673.

the ester group at C-6 and reglycosylation (with the same reagent) gave a resin to which a derivatized isomaltose was attached. The protecting groups were removed by standard methods, whereafter the disaccharide was cleaved photolytically from the polymer.

The Koenigs–Knorr reaction has continued to be used in the preparation of 'anomalous' 1,2-*cis*-related glycosides. Addition of silver salicylate increased the rate of reaction appreciably; thus, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and methanol in the presence of this salt gave the α-glycoside (84%) extremely rapidly at room temperature.[59] Isomaltose[60, 60a] and isomaltotriose,[60a] sterol α-D-glucosides,[61] 6-*O*-α-D-glucopyranosyl-D-galactose,[62] 6-*O*-α-D-glucopyranosyl-D-mannose,[60] and panose[60] have been prepared using various adaptations of the Koenigs–Knorr procedure. The use of 1-thioglycosides to give glycosyl halides and, thence, isomaltose, isomaltotriose, and isomaltotetraose is illustrated in Scheme 9.[63, 64]

6-Aminohexyl 2-acetamido-2-deoxy-β-D-glucopyranoside has been synthesized by standard methods, and has been used in modifying agarose for

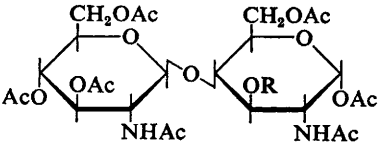


Reagents: i, $NO_2C_6H_4COCl$; ii, Br_2; iii, base; iv, repeat glycosylation; v, Pd–H_2

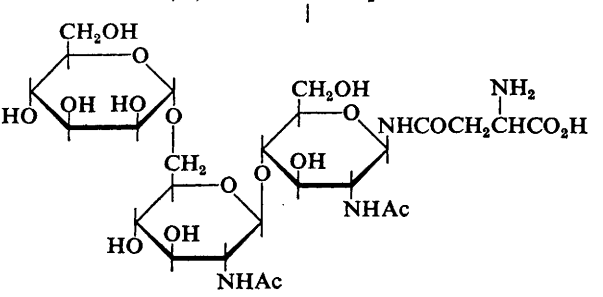
Scheme 9

[59] A. Ya. Veinberg, G. I. Roslovtseva, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1973, **43**, 688.
[60] B. Helferich and W. M. Müller, *Chem. Ber.*, 1973, **106**, 2508.
[60a] K. Takiura, K. Kakehi, and S. Honda, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 523.
[61] B. Helferich and W. M. Müller, *Chem. Ber.*, 1973, **106**, 715.
[62] B. Helferich and W. M. Müller, *Chem. Ber.*, 1973, **106**, 941.
[63] S. Koto, T. Uchida, and S. Zen, *Chem. Letters*, 1972, 1049 (*Chem. Abs.*, 1973, **78**, 30 112*r*).
[64] S. Koto, T. Uchida, and S. Zen, *Bull. Chem. Soc. Japan*, 1973, **46**, 2520.

affinity chromatography.[65] The disaccharide derivative (11) has been synthesized in order to confirm the structure of the repeating disaccharide unit obtained from the peptidoglycan released from bacterial-cell walls by the action of lysozyme.[66] The trisaccharide (12) has also been synthesized for similar reasons.[67]

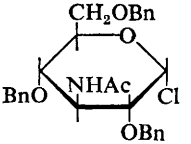


(11) R = $MeCHCO_2Me$

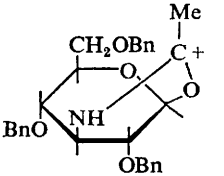


(12)

Japanese workers have reported on the reactions of glycosyl halides containing acetamidodeoxy-groups, and have shown that the solvent and the type of protecting group are important in determining the anomeric configuration of the glycosides produced.[68] With the halide (13), for example, conditions can be selected such that either β-glycosides are obtained by direct inversion, or α-glycosides are obtained by way of the intermediate ion (14). With the 6-acetamido-6-deoxy-isomer of the halide (13), α-compounds were found to predominate under all conditions and



(13)



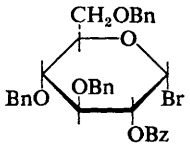
(14)

[65] R. Barker, K. W. Olsen, J. H. Shape, and R. L. Hill, *J. Biol. Chem.*, 1972, **247**, 7135.
[66] C. Merser and P. Sinaÿ, *Tetrahedron Letters*, 1973, 1029.
[67] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1973, **26**, 315.
[68] D. Nishimura, A. Hasegawa, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1767.

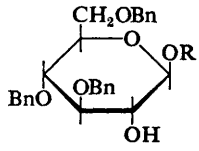
the 6-acetamido-group is considered to play a vital role in determining the stereospecificity of the reaction.

The reaction of various α-L-fucopyranosyl bromides with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside has been found to give ratios of anomeric disaccharides that depend on the nature of the protecting groups used.[69] The tri-*O*-acetate afforded only the β-linked disaccharide, by virtue of the participating role of the ester group at C-2, whereas the tri-*O*-benzyl ether gave a mixture of α- and β-linked disaccharides. However, the 2-*O*-benzyl-3,4-di-*O*-(*p*-nitrobenzoate) gave only the α-linked disaccharide, suggesting participation of the 3(4)-ester groups by way of six (seven)-membered acyloxonium ion intermediates. A specific method for the synthesis of β-D-mannopyranosides has utilized a Koenigs–Knorr reaction on the D-glucosyl bromide (15), followed by an oxidation–reduction sequence at C-2 of the β-D-glucopyranoside (16) obtained following treatment with base.[70]

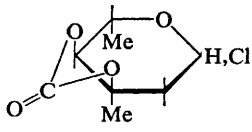
A more specific procedure yielding methyl 4-*O*-α- and 4-*O*-β-(L-mycarosyl)-β-D-mycaminosides involved condensation between compounds (17) and (18).[71]



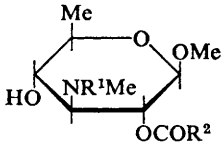
(15)



(16)



(17)



(18) $R^1 = CO_2Et$, $R^2 = PhN{=}NC_6H_4{-}$

Conventional Koenigs–Knorr syntheses have been used to establish that miserotoxin is 3-nitropropyl β-D-glucopyranoside,[72] and to obtain the β-D-glucopyranoside of 4-hydroxy-1,2-naphthoquinone (from 2-hydroxy-1,4-naphthoquinone),[73] a steroid derivative,[74] and glycosides of 2,4-dinitrophenol.[75] Various 3- and 21-linked sterol β-D-glucopyranosiduronic acids have also been reported.[76]

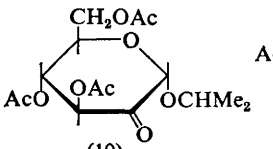
[69] M. Dejter-Juszynski and H. M. Flowers, *Carbohydrate Res.*, 1973, **30**, 287.
[70] G. Ekborg, B. Lindberg, and J. Lonngren, *Acta Chem. Scand.*, 1972, **26**, 3287.
[71] S. Koto, K. Yago, S. Zen, and S. Omura, *Chem. Letters*, 1972, 1091 (*Chem. Abs.*, 1973, **78**, 30 141z).
[72] H. H. Baer, S.-H. Lee Chiu, and D. C. Shields, *Canad. J. Chem.*, 1973, **51**, 2828.
[73] P. N. Cote and L. Goodman, *Carbohydrate Res.*, 1973, **26**, 247.
[74] H. Kubinyi, D. Hotz, and W. Steidle, *Annalen*, 1973, 224.
[75] F. Ballardie, B. Capon, J. D. G. Sutherland, D. Cocker, and M. Sinnott, *J.C.S. Perkin I*, 1973, 2418.
[76] V. R. Mattox and W. D. Vrieze, *J. Org. Chem.*, 1972, **37**, 3990.

The trisaccharides raffinose and its β-D-galactosyl isomer,[77] planteose,[77a] *p*-substituted-phenyl β-kojibiosides,[78] and various glycosylated 2-acetamido-2-deoxy-D-glucoses[79] have also been prepared by conventional methods.

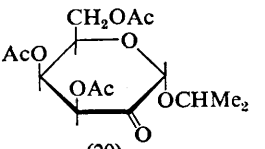
The related orthoester synthesis of glycosides has continued to be used extensively, and it has been shown that, as well as the expected acylated glycosides having the 1,2-*trans*-configuration, anomeric mixtures of the 2-hydroxy-analogues are produced as by-products.[80] A review of the synthesis of polysaccharides by this method has already been noted,[48] and specific applications of the method have included the preparations of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-β-D-mannopyranoside,[81] 3-*O*-β-D-glucopyranosyl-D-mannose,[82] *N*-acetyl-lactosamine,[83] 1-*O*-β-D-galactopyranosyl-D- and -L-ribitol,[84] various steroid and triterpenoid glycosides,[85] and 3-*O*-cellobiosyl-1,2-di-*O*-palmitoyl-*sn*-glycerol.[86] The orthoester procedure has been applied to the synthesis of gentiobiose using a solid support.[87]

Related methods using the established oxazoline procedure have been adopted to obtain glycosides and oligosaccharides of 2-acetamido-2-deoxy-β-D-galactopyranose.[88]

Unsaturated monosaccharide derivatives (see also Chapter 14) have continued to provide a useful means of preparing glycosides. Lemieux and his co-workers have extended their earlier work on the utilization of products obtained following the addition of nitrosyl chloride to glycals (see Vol. 2, Chapter 14). Detailed studies of the reduction of isopropyl 3,4,6-tri-*O*-acetyl-α-D-*arabino*- and -*lyxo*-hexopyranosid-2-uloses (19) and (20)



(19)



(20)

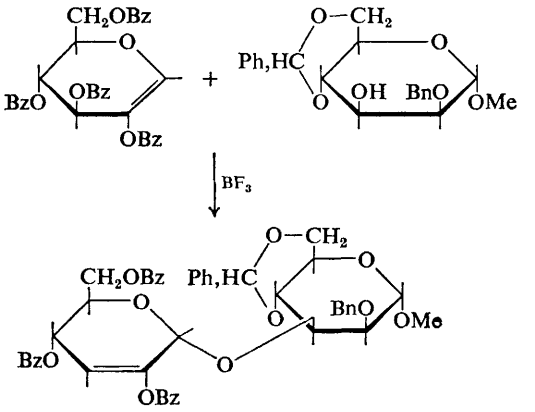
[77] T. Suami, T. Otake, T. Nishimura, and T. Ikeda, *Carbohydrate Res.*, 1973, **26**, 234.
[77a] T. Suami, T. Otake, T. Nishimura, and T. Ikeda, *Bull. Chem. Soc. Japan*, 1973, **46**, 1014.
[78] J. Duke, N. Little, and I. J. Goldstein, *Carbohydrate Res.*, 1973, **27**, 193.
[79] F. Schmitt and P. Sinaÿ, *Carbohydrate Res.*, 1973, **29**, 99.
[80] A. F. Bochkov, V. I. Betanely, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **30**, 418.
[81] P. J. Garegg and N.-H. Wallin, *Acta Chem. Scand.*, 1972, **26**, 3892.
[82] G. Alfredsson, H. B. Boren, and P. J. Garegg, *Acta Chem. Scand.*, 1972, **26**, 3431.
[83] S. E. Zarabyan, E. N. Lopantseva, and A. Ya. Khorlin, *Doklady Akad. Nauk S.S.S.R.*, 1973, **210**, 1216 (*Chem. Abs.*, 1973, **79**, 105 492*w*).
[84] P. J. Garegg, B. Lindberg, K. Nilsson, and C.-G. Swahn, *Acta Chem. Scand.*, 1973, **27**, 1595.
[85] N. I. Uvarova, G. I. Oshitok, and G. B. Elyakov, *Carbohydrate Res.*, 1973, **27**, 79.
[86] A. I. Bashkatova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1972, **8**, 2277.
[87] R. D. Guthrie, A. D. Jenkins, and G. A. F. Roberts, *J.C.S. Perkin I*, 1973, 2414.
[88] K. L. Matta, E. A. Johnson, and J. J. Barlow, *Carbohydrate Res.*, 1973, **26**, 215.

Table 1 *Products derived from reduction of isopropyl* 3,4,6-*tri*-O-*acetyl*-α-D-arabino-*hexopyranosid*-2-*ulose* (19) *and the* D-lyxo-*isomer* (20) (ref. 89)

	Product isopropyl 3,4,6-*tri*-O-*acetyl*-α-D-*hexopyranoside*			
Reducing agent	*gluco*	*manno*	*galacto*	*talo*
$NaBH_4$	96	4	60	40
B_2H_6	94	6	60	40
H_2,Pd/C	32	68	9	91
H_2,Ru/C	40	60	20	80
H_2,Rh/C	20	80	15	85
H_2,PtO_2	75	25	13	87
H_2,Ni	60	40	17	83

were carried out with the results shown in Table 1.[89] Thus, highly selective reductions can be achieved, and the potential of the method was exemplified by syntheses of 2-*O*-α-D-mannopyranosyl-glycerol and the related D-*gluco*-isomer. A related report has described syntheses of 6-*O*-α-D-glucopyranosyl-D-galactose and α-D-galactopyranosyl and α-D-talopyranosyl analogues thereof, in addition to the 3-linked hexopyranosyl D-glucoses.[90] Analogous reductions of appropriate 2-oximo-derivatives were used to obtain 2-acetamido-2-deoxy-α-D-glucopyranosyl, -galactopyranosyl, and -talopyranosyl derivatives of D-galactose (6-linked) and D-glucose (3-linked).[91]

The hydroxyglycal method (Vol. 3, p. 112) has been used (Scheme 10) for the synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside, which was required for use in immunological studies. The condensation step was followed by hydrogenation and by introduction

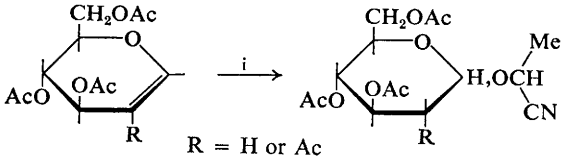


Scheme 10

[89] R. U. Lemieux, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 27.
[90] R. U. Lemieux, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 42.
[91] R. U. Lemieux, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 48.

of the 6′-deoxy-function by standard transformations.[92] (It should be noted that errors in the formulae appearing in this paper have been corrected [92a].)

Photochemical addition of acetaldehyde cyanohydrin to 3,4,6-tri-*O*-acetyl-D-glucal or its 2-acetoxy-derivative gave the glycosidic products indicated in Scheme 11, but the stereoselectivity of the addition is low.[93]



Reagent: i, MeCH(OH)CN–*hν*

Scheme 11

Methoxymercuration of 3,4,6-tri-*O*-acetyl-D-glucal has been used to prepare 2-deoxy-disaccharides (see Chapter 14).

Syntheses of the following glycosides have also been reported: azidophenyl [94] and diazoacetamidophenyl [95] glycosides (for use in enzymic studies), 2-*O*-β-D-glucopyranosyl-1,4-dithio-D-threitol (by enzymic methods),[96] phenyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (by standard procedures),[97] and *p*-nitrophenyl and *p*-aminophenyl 2-acetamido-2-deoxy-β-D-galactopyranosides (from the corresponding D-glucopyranoside by use of nucleophilic displacements).[98]

An improved procedure for obtaining methyl α-D-altropyranoside is referred to in Chapter 5.

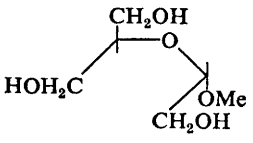
Hydrolysis and Related Reactions.—Acid-catalysed hydrolyses have continued to receive attention. 4-*O*-Alkyl-α- and -β-D-glucopyranosides have been found to hydrolyse more slowly than the unsubstituted analogues, a finding that has important implications on the behaviour of polysaccharides towards acid.[99] Related studies on thirty-two *o*-, *m*-, and *p*-substituted-phenyl β-D-galactopyranosides showed that only electronic effects influence the rates of reaction.[100] Interesting work with 1-adamantyl β-D-glucopyranoside and t-butyl, 1,1-diethylpropyl, and diphenylmethyl

[92] G. Alfredsson and P. J. Garegg, *Acta Chem. Scand.*, 1973, **27**, 556.
[92a] G. Alfredsson and P. J. Garegg, *Acta Chem. Scand.*, 1973, **27**, 1834.
[93] K. Matsuura, Y. Araki, Y. Ishido, and M. Kainosho, *Chem. Letters*, 1972, 853 (*Chem. Abs.*, 1973, **78**, 30 105*c*).
[94] E. Saman, M. Claeyssens, H. Kersters-Hilderson, and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **30**, 207.
[95] E. W. Thomas, *Carbohydrate Res.*, 1973, **31**, 101.
[96] D. L. Storm, R. C. Buri, and W. Z. Hassid, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 147.
[97] I. Dijong and F. Werner, *Carbohydrate Res.*, 1973, **27**, 273.
[98] M. Petitou and P. Sinaÿ, *Carbohydrate Res.*, 1973, **29**, 502.
[99] J. N. BeMiller and E. R. Doyle, *Carbohydrate Res.*, 1972, **25**, 429.
[100] C. K. de Bruyne, J. Wouters-Leysen, and M. Yde, *Carbohydrate Res.*, 1973, **29**, 387.

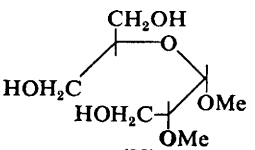
β-D-galactopyranosides has led to the conclusions that the tertiary glycosides have considerable F-strain which can be relieved by either alkyl–oxygen or glycosyl–oxygen bond-scission, thus accounting for the accelerated rates observed, and that alkyl–oxygen bond-scission of non-bridgehead tertiary glycosides is predominant in the D-glucoside series and is significant in the D-galactoside series.[101]

The acid- and enzyme-catalysed hydrolyses of the methyl glycosides of *N*-acetylneuraminic acid and its tetra-acetate have shown that esterification is responsible for the previously observed resistance towards hydrolysis shown by acetylated compounds of this type.[102] In related work, it has been found that *p*-nitrophenyl 2-*O*-acetyl-β-D-glucopyranoside is not hydrolysed by *N*-acetyl-β-D-glucosaminidase, demonstrating the necessity of the 2-acetamido-group for the manifestation of enzymic activity.[103]

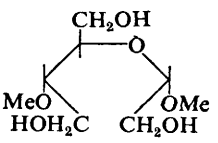
An interesting study on the relative rates of hydrolysis of methyl α-D-glucopyranoside and the acyclic acetals (21)—(23), which are models for



(21)



(22)



(23)

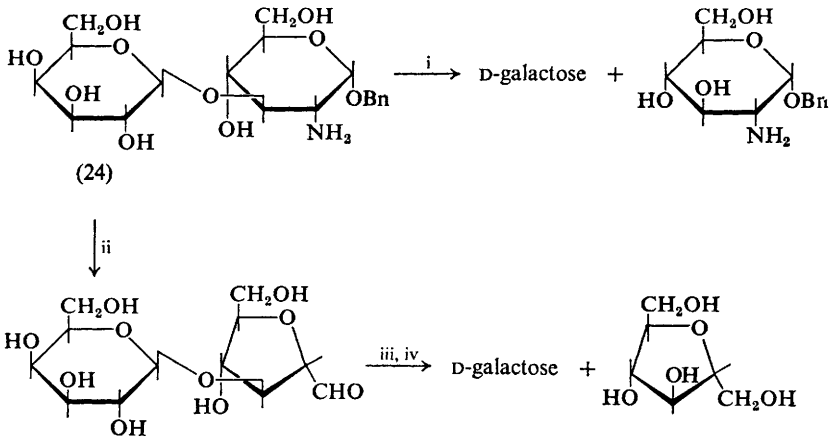
components of periodate-oxidized and reduced polysaccharides, has revealed that the oxidized units should be hydrolysable under conditions that do not affect the unoxidized units.[104] Compounds (21) and (23) were hydrolysed at approximately the same rate, compound (22) reacted ten times more slowly, and the relative rate of hydrolysis of the cyclic methyl glycoside was significantly slower by a factor of 2×10^4.

Russian workers have examined various aspects of acetolytic reactions: disaccharide linkages were cleaved in the order (1 → 1) > (1 → 6) > (1 → 3) > (1 → 4) > (1 → 2), and D-galactosyl compounds were more reactive than D-glucosyl analogues.[105] Aldobi-itols and aldobionic acids containing (1 → 2)-linkages were cleaved more readily than the parent disaccharides, but cleavage occurred more slowly when either (1 → 4)- or (1 → 6)-linkages were involved.[106] For aldobiouronic acids, the carboxy-group was found to stabilize a (1 → 4)-linkage more than a (1 → 6)-linkage, whereas the carboxy-group in the reducing moiety of pseudoaldobiouronic acids had no effect on the stability of the intersaccharide bonds.[107]

[101] D. Cocker, L. E. Jukes, and M. L. Sinnott, *J.C.S. Perkin II*, 1973, 190.
[102] A. Neuberger and W. A. Ratcliffe, *Biochem. J.*, 1973, **133**, 623.
[103] K. Yamamoto, *Bull. Chem. Soc. Japan*, 1973, **46**, 290.
[104] B. Erbing, O. Larm, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 1094.
[105] V. I. Govorchenko and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, 256.
[106] V. I. Govorchenko, V. I. Gorbatch, and Yu. S. Ovodov, *Carbohydrate Res.*, 1973, **29**, 421.
[107] V. I. Govorchenko, V. I. Gorbach, and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, 258.

The degradation of sucrose with alkali has been investigated in an effort to learn about a process that results in loss of the sugar during its commercial preparation, and that could conceivably lead to a convenient preparation of lactic acid.[108] Octa-*O*-methylsucrose was stable under conditions (1M-NaOH at 100 °C) that degraded the unsubstituted disaccharide, implying that intramolecular reactions of oxyanions are involved in the degradation. It was suggested that the hydroxy-groups of both D-fructosyl and D-glucosyl moieties could be involved in the generation of these anions, and that an 'inter-unit' process is involved, since methyl α-D-glucopyranoside and methyl β-D-fructofuranoside were unreactive. More-detailed studies suggested that the C-3 hydroxy-group of the D-fructosyl moiety might be primarily responsible for the intramolecular reaction.

Studies on the selective cleavage of glycosidic linkages in polysaccharides containing 2-amino-2-deoxyhexose residues have been undertaken using the disaccharide (24) as a model (see Scheme 12).[109] A related investigation



Reagents: i, HCl; ii, HNO_2; iii, KBH_4; iv, H^+

Scheme 12

with benzyl 2-amino-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside has been reported.[110]

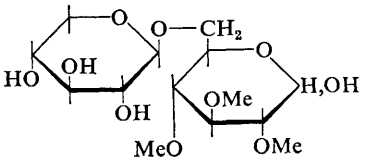
Studies with photolysable glycosides have been referred to already,[58] and it has also been reported that u.v. light readily cleaved the glycosidic linkages in various triterpenoid saponins.[111] However, it is not clear

[108] G. W. O'Donnell and G. N. Richards, *Austral. J. Chem.*, 1973, **26**, 2041.
[109] B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **29**, 451.
[110] B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **30**, 45.
[111] I. Kitigawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. and Ind.*, 1973, 276.

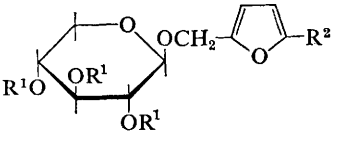
which parts of the molecules act as photon-acceptors using radiation from a high-pressure mercury lamp. Photochemical cleavage of a uronoside is mentioned in Chapter 17.

N.m.r. studies on glycosidic compounds are described in Chapter 23.

Other Reactions and Features of Glycosides.—In connection with studies on the base-catalysed degradation of components of polysaccharides, Aspinall and his co-workers have shown that the tri-*O*-methyl-disaccharide (25) is degraded to the furan derivative (26) on treatment with lime water, followed



(25)



(26) R^1 = H, R^2 = CHO
(27) R^1 = Ac, R^2 = CO_2Me

by acid; in the same way, 2,3,4,6-tetra-*O*-methyl-D-glucose has been shown to give 5-methoxymethyl-2-furaldehyde.[112] The structure of the product (26) was confirmed by its conversion into the furan ester (27), which was synthesized independently.

Pyrolyses of phenyl α- and β-D-glucopyranosides in the presence and in the absence of sodium hydroxide have been investigated by chemical and physical methods.[113] As in aqueous systems, the β-anomer was converted more readily into 1,6-anhydro-β-D-glucopyranose. The same group have also reported on the thermal analysis of variously substituted phenyl glycosides; differential thermal, thermogravimetric, and derivative thermogravimetric analytical curves were illustrated for this series of compounds.[114] It was found that pyrolytic cleavage of glycosidic bonds is considerably retarded by acetylation of the carbohydrate moieties, and that it is also affected by the inductive effects of substituents on both phenolic and carbohydrate moieties; electron-withdrawing groups facilitated pyrolytic cleavage. In a continuation of this work, several types of transition that precede the melting of crystalline carbohydrates (aryl glycosides and various esters and anhydrides) have been investigated by thermal analysis.[115]

A ring-contraction was observed when methyl β-D-arabinopyranoside triacetate was treated with hydrogen bromide (see Chapter 7).

A number of reports on biological aspects of glycosides have been published. The binding of *p*-substituted-phenyl glycopyranosides of α-D-glucose, β-D-glucose, and α-D-mannose to concanavalin A has been

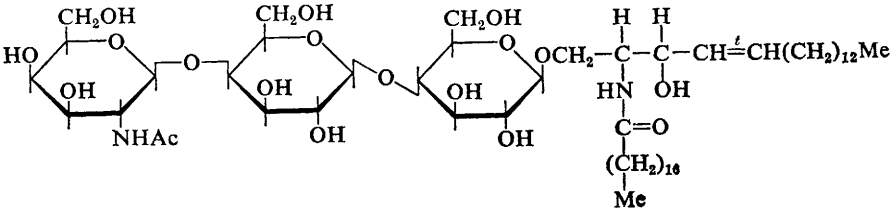
[112] G. O. Aspinall, R. Khan, R. R. King, and Z. Pawlak, *Canad. J. Chem.*, 1973, **51**, 1359.
[113] F. Shafizadeh, Y. Z. Lai, and R. A. Susott, *Carbohydrate Res.*, 1972, **25**, 387.
[114] F. Shafizadeh, M. H. Meshreki, and R. A. Susott, *J. Org. Chem.*, 1973, **38**, 1190.
[115] F. Shafizadeh and R. A. Susott, *J. Org. Chem.*, 1973, **38**, 3710.

related to the electronic and hydrophobic characteristics of the substituents,[116] and a related paper has described the binding of *p*-nitrophenyl α-D-mannopyranoside specifically.[117] The effects of *p*-substitution on the behaviour of phenyl α-D-mannopyranosides as specific ligands for lectins from peas and lentils have also been examined.[118]

The inhibition by sterols of the enzymic D-glucosylation of *p*-nitrophenol has been studied as part of an investigation on steroidal conjugation.[118a]

The ^{1}H n.m.r. spectra of a number of acetylated steroid and triterpenoid glycosides have been reported.[119]

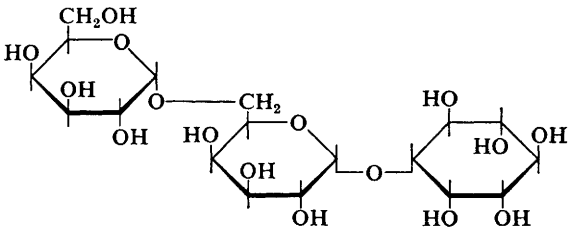
Natural Products.—The following di- and tri-saccharides have been found in Nature during 1973; 3-*O*-α-D-xylopyranosyl- and 2-*O*-α-L-fucopyranosyl-D-glucoses (from human urine),[120] *O*-α-D-mannopyranosyl-(1 → 3)-*O*-β-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose (from the urine of patients with mannosidosis),[121] and 6-deoxy-4-*O*-(6-deoxy-α-D-glucopyranosyl)-D-glucose,[122] 6-deoxy-4-*O*-(6-deoxy-α-D-galactopyranosyl)-D-glucose, and 6-deoxy-4-*O*-(6-deoxy-α-D-galactopyranosyl)-D-galactose (in hydrolysates of asterosaponin).[123] Micro-organisms have yielded the trisaccharides 3-*O*-β-gentiobiosyl-D-glucose[124] and *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 6)-D-galactose,[125] and the Tay–Sachs' trisaccharide glycoside (28) has been isolated, characterized, and synthesized.[126]



(28)

[116] F. G. Loontiens, J. P. Van Wauwe, R. De Gussem, and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **30**, 51.
[117] R. D. Gray and R. H. Glew, *J. Biol. Chem.*, 1973, **248**, 7547.
[118] J. P. Van Wauwe, F. G. Loontiens, H. A. Carchon, and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **30**, 249.
[118a] T. Gessner, A. Jacknowitz, and C. A. Vollmer, *Biochem. J.*, 1973, **132**, 249.
[119] A. K. Dzizenko, V. V. Isakov, N. I. Uvarova, G. I. Oshitok, and G. B. Elyakov, *Carbohydrate Res.*, 1973, **27**, 249.
[120] A. Lundblad and S. Svensson, *Biochemistry*, 1973, **12**, 306.
[121] N. E. Norden, A. Lundblad, S. S. Svensson, P.-A. Ockerman, and S. Autio, *J. Biol. Chem.*, 1973, **248**, 6210.
[122] S. Ikegami, Y. Hirose, Y. Kamiya, and S. Tamura, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2449.
[123] S. Ikegami, Y. Hirose, Y. Kamiya, and S. Tamura, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2453.
[124] Y. Ueno and M. Kitahara, *Carbohydrate Res.*, 1973, **28**, 140.
[125] F. Pratviel-Sosa, R. Wylde, R. Bourbouze, and F. Percheron, *Carbohydrate Res.*, 1973, **28**, 109.
[126] D. Shapiro, A. J. Acher, and Y. Rabinsohn, *Chem. and Phys. Lipids*, 1973, **10**, 28.

Other glycosides of interest to be reported were the digalactosyl-*myo*-inositol (29) (from rape-seed meal),[127] antigenin-4′-β-gentiobioside (from a micro-organism),[128] a steroidal 6-deoxy-β-D-glucopyranoside (from star-fish),[129] and new flavonoid glycosides.[130–134]



(29)

Hydrolysis of naturally occurring cardiac glycosides has yielded two new disaccharides, which were identified as 6-deoxy-4-*O*-β-D-glucopyranosyl-D-gulose (erikordinobiose) and 2,6-dideoxy-4-*O*-β-D-xylopyranosyl-D-*ribo*-hexose (erikhrobiose).[135] Nine new glycosides of oleanolic acid, containing from one to three sugar residues, have been isolated from the roots of *Calendula officinalis*.[136]

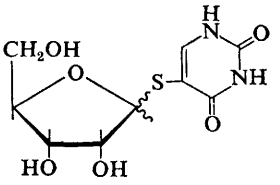
It should be noted that, as in previous volumes, no attempt has been made to offer comprehensive coverage in this area.

S- and *Se*-Glycosides

A new synthesis of 1-thio-D-glucosides is reported in Chapter 14, and reference has been made already to the use of 1-thioglycosides in the synthesis of *O*-glycosides. 1-Thioglycosides have been prepared by the photochemical addition of thiols to hydroxyglycal esters (see Chapter 14) and by treatment of acylglycosyl halides with lead dialkylthiols.[137]

The following 1-thioglycosides have been prepared: *p*-substituted-phenyl 1-thio-β-D-galactopyranosides,[138] alkyl 1-thio-β-D-galactopyranosides (by alkylation of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose),[139] and

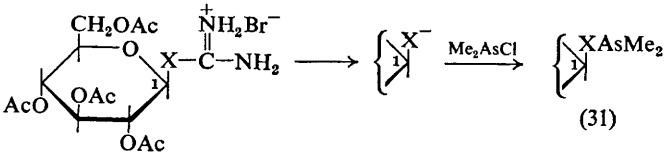
[127] I. R. Siddiqui, P. J. Wood, and G. Khanzada, *Carbohydrate Res.*, 1973, **29**, 255.
[128] S. Nishibe, S. Hisada, and I. Inagaki, *Experientia*, 1973, **29**, 17.
[129] Y. M. Sheikh and C. Djerassi, *Tetrahedron Letters*, 1973, 2927.
[130] N. A. Tsepkova, A. N. Svechnikova, Y. A. Bandjukova, and Kh. Kh. Khalmatov, *Khim. prirod. Soedinenii*, 1972, 661.
[131] V. I. Bykov and V. I. Glyzin, *Khim. prirod. Soedinenii*, 1972, 672.
[132] E. V. Gella, V. I. Vavilov, and N. G. Ermolov, *Khim. prirod. Soedinenii*, 1972, 674.
[133] V. I. Bykov, V. I. Glyzin, and A. I. Bankovsky, *Khim. prirod. Soedinenii*, 1972, 715.
[134] N. Sh. Kattaev, I. A. Kharlamov, N. M. Akhmedkhodjaeva, G. K. Nikonov, and Kh. Kh. Khalmatov, *Khim. prirod. Soedinenii*, 1972, 806.
[135] I. F. Makarevich, *Khim. prirod. Soedinenii*, 1973, 50.
[136] L. P. Vecherko, E. P. Zinkevich, and A. F. Sviridov, Abstracts 3rd Soviet–Indian Symposium Chem. Natural Products, Tashkent, 1973, p. 48.
[137] H. M. A. Abdel-Bary, F. M. E. Abdel-Megeid, Z. El-Hewehi, and M. A. F. Elkaschef, *J. prakt. Chem.*, 1972, **314**, 461.
[138] M. Yde and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **26**, 227.
[139] M. Yde and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **30**, 205.



(30)

benzyl, *p*-nitrobenzyl, and *p*-aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside.[140] The α- and β-anomers of the uracil thioglycoside (30) have also been reported.[141]

New types of carbohydrate derivative containing Me_2AsS- and Me_2AsSe-groups as the glycosyl substituents have been obtained, as indicated in Scheme 13. The D-glucosyl thioarsenous compound was obtained by



(31)

X = S or Se

Scheme 13

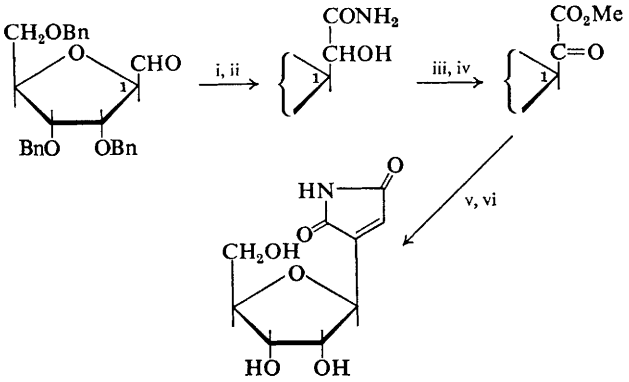
deacetylation of the ester (31; X = S), and the corresponding seleno-compound (31; X = Se) by treatment of di-(β-D-glucopyranosyl)di-selenide with tetramethyldiarsine (Me_4As_2).[142]

C-Glycosides

A number of *C*-glycosides of biological interest have been synthesized during the past year.

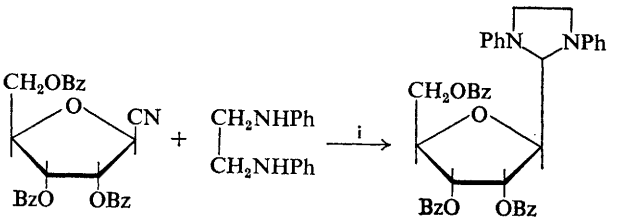
An elegant synthesis (Scheme 14) of showdomycin has been reported from Moffatt's laboratory,[143] and the same group have advised on the synthesis of derivatives of 2,5-anhydro-D-allose (Scheme 15);[144] without trapping of the intermediate aldehyde by the diamine in the latter synthesis, 5-benzoyloxy-2-furaldehyde was obtained. Mild acidic hydrolysis of the imidazolidine afforded the aldehydic compound, which was required for use in the synthesis of *C*-linked β-D-ribofuranosyl nucleosides (for example, see Scheme 14). Reference is made to closely related 2,5-anhydro-sugars in the appropriate section of Chapter 4, and additions to glycals have also afforded *C*-glycosides (see Chapter 14).

[140] K. L. Matta, E. A. Z. Johnson, R. N. Girotra, and J. N. Barlow, *Carbohydrate Res.*, 1973, **30**, 414.
[141] G. L. Szekeres and T. J. Bardos, *J. Medicin. Chem.*, 1972, **15**, 1333.
[142] R. A. Zingaro and J. K. Thomson, *Carbohydrate Res.*, 1973, **29**, 147.
[143] G. Trummlitz and J. G. Moffatt, *J. Org. Chem.*, 1973, **38**, 1841.
[144] H. P. Albrecht, D. B. Repke, and J. G. Moffat, *J. Org. Chem.*, 1973, **38**, 1836.



Reagents: i, NaCN; ii, H_2O_2; iii, MeOH–H^+; iv, DCC–DMSO; v, $Ph_3P{=}CHCONH_2$; vi, BCl_3 (−78 °C)

Scheme 14

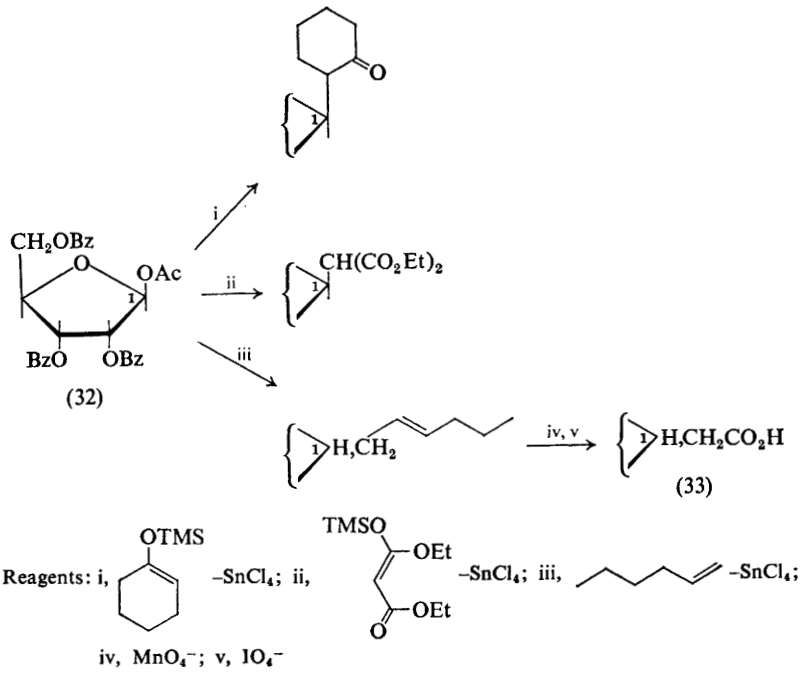


Reagent: i, Ni–NaH_2PO_2–AcOH–py–H_2O

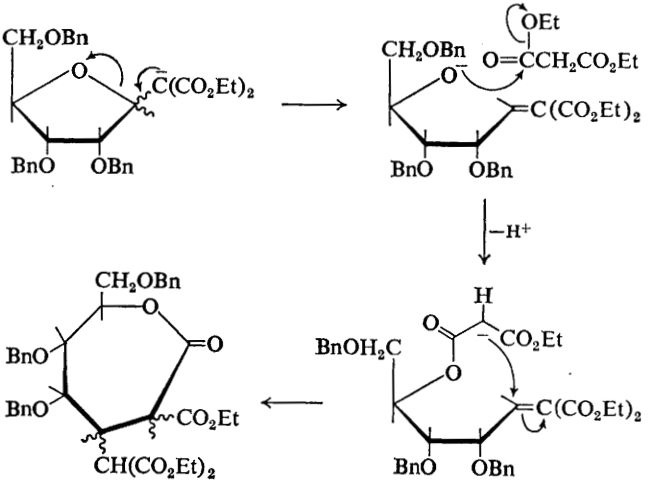
Scheme 15

Hanessian and his group have continued their studies in this field. Condensation of enol trimethylsilyl ethers with the glycosyl acetate (32) yielded *C*-glycosides, as shown in Scheme 16, and the same acetate was allowed to react with an olefin to provide a new route to the glycosyl acetic acids (33).[145] It was also found that reaction of 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl chloride with sodio diethylmalonate gave an anomeric mixture of the expected glycosylated diethyl malonates;[146] similar treatment of the corresponding bromide gave a ring-expanded product, presumably by the route shown in Scheme 17. This paper also referred to the base-catalysed anomerization of glycosyl diethyl malonates; Ohrui and Fox have noted similar anomerizations and have synthesized the more stable β-*C*-nucleoside (34) by treatment of the mixture of anomers with base.[147]

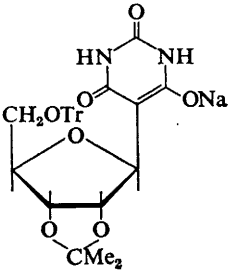
[145] T. Ogawa, A. G. Pernet, and S. Hanessian, *Tetrahedron Letters*, 1973, 3543.
[146] A. G. Pernet, T. Ogawa, and S. Hanessian, *Tetrahedron Letters*, 1973, 3547.
[147] H. Ohrui and J. J. Fox, *Tetrahedron Letters*, 1973, 1951.



Scheme 16



Scheme 17



(34)

The reaction between 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide and various di- and tri-methoxybenzenes furnished *C*-glycosylated benzene derivatives that were then used in the preparation of analogous benzoquinone derivatives.[148]

[148] L. Kalvoda, *Coll. Czech. Chem. Comm.*, 1973, **38**, 1679.

4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—A comparative study of various methylation techniques applied to methyl β-D-xylopyranoside has given the relative reactivities of the three hydroxy-groups shown in Table 2.[149]

Table 2 *Methylation of methyl β-D-xylopyranoside*

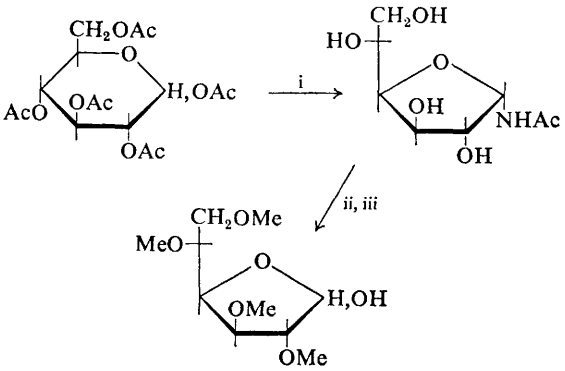
Reagent	*Name of reaction*	*Order of reactivity of hydroxy-groups*
$NaOH–Me_2SO_4–H_2O$	Haworth	2 > 4 > 3
Ag_2O–MeI–MeOH	Purdie	2 > 4 > 3
Ag_2O–MeI–DMF	Kuhn	2 > 4 > 3
$Na^+ {}^-CH_2SOMe$–MeI–DMSO	Hakomori	4 > 2 > 3

4-*O*-Methyl-D-xylose, 3-*O*-methyl-D-mannose, and 6-deoxy-3-*O*-methyl-D-talose have been characterized among the products of hydrolysis of the polysaccharides extracted from two Gram-negative bacteria.[150] Mass spectrometry, electrophoresis, and paper chromatography were the main analytical tools used in determining the structures of these sugars.

Methyl ethers of D-glucose have continued to attract attention. The 4-*O*-methyl ether (and the benzyl analogue) has been synthesized using methyl 2,3-di-*O*-allyl-6-*O*-triphenylmethyl-α-D-glucopyranoside as the key intermediate,[151] and the 6-*O*-methyl ether has been obtained by methylation of α-D-glucofuranose 1,2:3,5-bis(phenylboronate) using diazomethane–boron trifluoride.[152] Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside has been converted into the 2,4,6-tri-*O*-methyl ether,[153] and 2,3,5,6-tetra-*O*-methyl-D-glucofuranose has been obtained as shown in Scheme 18.[154] Complete methylation of D-glucose, D-galactose, and acetylated derivatives thereof has been referred to already.[55]

On treatment with alkali, 2,3,4,6-tetra-*O*-methyl-D-glucose afforded a 3-deoxyhex-2-enopyranosyl derivative, some reactions of which are described in Chapter 14.

[149] Yu. S. Ovodov and E. V. Tushenko, *Carbohydrate Res.*, 1973, **27**, 169.
[150] J. Weckesser, H. Mayer, and I. Fromme, *Biochem. J.*, 1973, **135**, 293.
[151] J. W. Van Cleve and C. R. Russell, *Carbohydrate Res.*, 1972, **25**, 465.
[152] E. J. Bourne, I. R. McKinley, and H. Weigel, *Carbohydrate Res.*, 1972, **25**, 516.
[153] P. Kovac and Z. Longaverova, *Chem. Zvesti*, 1973, **27**, 415 (*Chem. Abs.*, 1973, **79**, 92 535*k*).
[154] M. E. Gelpi and R. A. Cadenas, *Carbohydrate Res.*, 1973, **28**, 147.

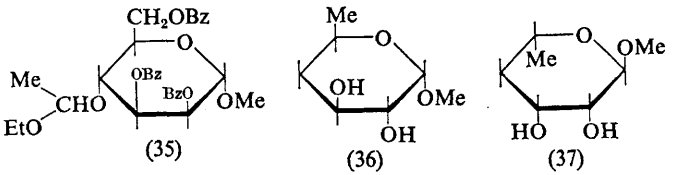


Reagents: i, NH_3–H_2O; ii, MeI–BaO; iii, H^+

Scheme 18

In the D-galactose series, convenient syntheses of the 2- and 3-*O*-methyl ethers have been described by way of methylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside 3- and 2-benzoates, respectively, using diazomethane,[155] and the 3,6-di-*O*-methyl ether has been prepared by a multi-stage procedure from 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose.[156]

The 4-*O*- and 2,4-di-*O*-methyl ethers of D-mannose have been obtained by methylation of the appropriate benzoates, and the 2,3,6-tri-*O*-methyl ether was synthesized from the acetalated derivative (35).[157] Standard methods have been used to obtain 3-, 4-, 3,4-di-, and 3,4,6-tri-*O*-methyl ethers of methyl 2-acetamido-2-deoxy-α-D-mannopyranoside.[158]



(35) (36) (37)

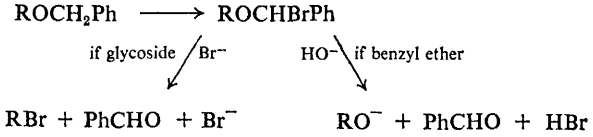
Partial methylation of the methyl 4,6-dideoxyhexosides (36) and (37) with methyl iodide and sodium hydroxide in DMF gave results as follows (respective percentages): starting materials, 17, 16%; 2-ethers, 38, 29%; 3-ethers, 10, 13%; 2,3-diethers, 26, 15%.[159]

1-*O*-Methyl-D-erythritol, 1,4-di-*O*-methylerythritol, and the corresponding methyl ethers of L-threitol have been reported.[159a]

Substituted Alkyl and Aryl Ethers.—Benzyl 3-*O*-allyl-α-D-glucopyranoside has been used as the starting material for syntheses of the 2,4-di- and

[155] G. J. F. Chittenden, *Carbohydrate Res.*, 1973, **31**, 127.
[156] A. Penman and D. A. Rees, *J.C.S. Perkin I*, 1973, 2188.
[157] F. R. Seymour, *Carbohydrate Res.*, 1973, **30**, 327.
[158] Nasir-Ud-Din and R. W. Jeanloz, *Carbohydrate Res.*, 1973, **28**, 243.
[159] K. Kefurt, Z. Kefurtová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1973, **38**, 2627.
[159a] P. Nanasi and A. Liptak, *Carbohydrate Res.*, 1973, **29**, 201.

2,4,6-tri-*O*-benzyl derivatives,[160] and benzyl 2,4-di-*O*-benzyl-β-D-galactopyranoside has been obtained by way of the 3,6-di-*O*-methanesulphonate.[161] Partial benzylation of methyl α-L-fucopyranoside furnished a mixture of 2,4- and 3,4-disubstituted products, whereas the 2,4- and 2,3-disubstituted isomers were obtained by partial benzylation of the 2-*O*-benzylglycoside. The disubstituted derivatives were then used to prepare 2-, 3-, and 4-*O*-methyl-L-fucoses.[162] Following earlier work on debenzylation by free-radical α-bromination and hydrolysis (*J. Org. Chem.*, 1968, **33**, 4292), it has been shown (Scheme 19) that the reaction proceeds as expected for normal



Scheme 19

ethers, but that glycosidic benzyl ethers (*i.e.* acetals) afford glycosyl bromides.[163]

The 2-methylallyl ether group has been found to isomerize more slowly than the allyl and but-2-enyl ether groups, which can be removed selectively in its presence.[164]

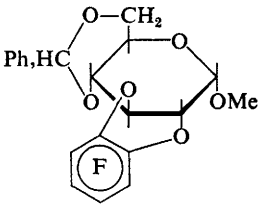
Vinyl ethers of 1,6-anhydro-β-D-glucopyranose[165] and 1,4:3,6-dianhydro-D-glucitol and -mannitol[166] have been reported, and the latter compounds were shown to polymerize in the presence of boron trifluoride. 3,5,6-Tri-*O*-allyl-D-glucofuranose has been prepared and reduced to the tri-*O*-propyl ether, and the former compound was also polymerized.[167]

Rats fed on hydroxypropylated potato starches have been shown to excrete 2′-*O*-(2-hydroxypropyl)-[168] and 6′-*O*-(2-hydroxypropyl)-maltose.[169]

Tritylpyridinium fluoroborate (prepared from pyridine and trityl fluoroborate and used in acetonitrile solution) has been recommended for the tritylation of primary hydroxy-groups.[170]

[160] T. Lakhanisky and H. P. Neveau, *Cellulose Chem. Technol.*, 1972, **6**, 127 (*Chem. Abs.*, 1973, **78**, 84 673*t*).
[161] S. David, C. A. Johnson, and A. Veyrieres, *Carbohydrate Res.*, 1973, **28**, 121.
[162] M. Dejter-Juszynski and H. M. Flowers, *Carbohydrate Res.*, 1973, **28**, 61.
[163] J. N. BeMiller and H. L. Muenchow, *Carbohydrate Res.*, 1973, **28**, 253.
[164] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1973, 1858.
[165] E. Yu. Ponomarenko, V. L. Lapenko, and G. G. Markova, *Trudy Vironezhsk. Gosud Univ.*, 1972, **95**, 72 (*Chem. Abs.*, 1973, **78**, 4437*f*).
[166] B. I. Mikhant'ev, V. L. Lapenko, and A. I. Slivkin, *Zhur. obshchei Khim.*, 1972, **42**, 2302.
[167] A. I. Slivkin, *Sb. Stud. Nauchn. Rabot. Voronezhsk. Gosud. Univ.*, 1970, 69 (*Chem. Abs.*, 1973, **78**, 136 545*z*).
[168] D. C. Leegwater, M. C. Ten Noever De Brauw, A. Mackor, and J. W. Marsman, *Carbohydrate Res.*, 1972, **25**, 411.
[169] D. C. Leegwater and J. W. Marsman, *Carbohydrate Res.*, 1973, **29**, 271.
[170] S. Hanessian and A. P. A. Staub, *Tetrahedron Letters*, 1973, 3555.

Interesting perfluorophenyl ethers of carbohydrates have been prepared from isolated hydroxy-groups by generation of the oxyanion, followed by treatment with hexafluorobenzene in 1,2-dimethoxyethane. If the mono-ether of a 1,2-diol was further treated with sodium hydride, a cyclic diether [*e.g.* (38)] resulted.[171]



(38)

Silyl Ethers.—t-Butyldimethylsilyl ethers of nucleosides have been prepared, and their stabilities were examined under a variety of conditions (see also Chapter 21). Not unexpectedly, ethers at the primary position were formed most readily.[172]

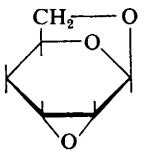
The trimethylsilylation of 2-amino-2-deoxyhexoses has been studied and conditions have been found for substitution of one or both hydrogen atoms of the amino-groups; g.l.c. was used to examine the products.[173]

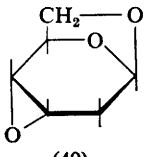
Intramolecular Ethers (Anhydro-sugars)

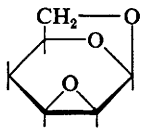
Epoxides.—Anion-exchange resins have been used in the preparation of various water-soluble epoxides from 1,6-anhydrohexose tosylates.[174]

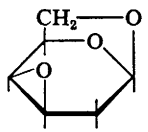
A number of cyclohexene oxides having oxygenated functions adjacent to the oxiran ring have been opened with lithium aluminium hydride. The formation of stereochemically anomalous products was discussed, and the findings are relevant to analogous reactions of pyranoside epoxides.[175] A related study in the carbohydrate field has examined epoxide-ring openings (with ^{-}OH, MgI_2,MeMgI, $LiAlH_4$, and H_2–Ni) of the *ribo*-dianhydrides (39) and (40) and the *lyxo*-isomers (41) and (42).[176] Whereas the former pair gave products of diaxial ring-opening, both diaxial and diequatorial products were obtained from the *lyxo*-dianhydrides. The results were interpreted in terms of both steric and polar effects, and it was concluded that the latter effects may play a significant part in determining the mode of opening of oxiran rings. Opening of the oxiran rings of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allo- and -manno-pyranosides with lithium dimethyl cuprate gave the 2-deoxy-2-*C*-methyl- and 3-deoxy-3-*C*-methyl-D-altropyranosides, respectively, which were then converted

[171] A. H. Haines and K. C. Symes, *J.C.S. Perkin I*, 1973, 53.
[172] K. K. Ogilvie and D. J. Iwacha, *Tetrahedron Letters*, 1973, 317.
[173] R. E. Hurst, *Carbohydrate Res.*, 1973, **30**, 143.
[174] J. Stanek, jun. and M. Černý, *Synthesis*, 1972, 698.
[175] B. C. Hartman and B. Rickborn, *J. Org. Chem.*, 1972, **37**, 4246.
[176] J. Halbych, T. Trnka, and M. Černý, *Coll. Czech. Chem. Comm.*, 1973, **38**, 2151.


(39)


(40)


(41)


(42)

into the corresponding 2-*C*-methyl- and 3-*C*-methyl-2,3-dideoxyhex-2-enosides by means of the Chugaev reaction (see Chapter 14).[177]

Peroxy-acid oxidation of 1-*O*-acetyl-3-deoxy-2-*O*-methyl-hex-2-enopyranoses gave 2,3-epoxides, in addition to orthoesters, as products (see Chapter 14).[178, 178a] Analogous furanosyl compounds have been readily obtained both from nucleoside 2,3-orthoesters by way of 3-halogeno-intermediates,[179] and from unsubstituted nucleosides by a two-step process (see Chapter 7). A rearrangement of Brigl's anhydride is described in the following section.

Sugar epoxides are also referred to in Chapters 6, 7, and 11.

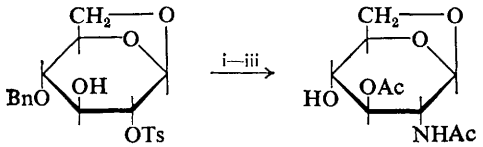
Other Anhydrides.—The polymerization of derivatives of 1,6-anhydrohexoses to produce synthetic polysaccharides has been reviewed,[48] and it has been found that 1,6-anhydro-2,3,5-tri-*O*-benzyl-α-D-galactofuranose polymerized on treatment with phosphorus pentafluoride at low temperatures to give mainly a β-linked polymer.[180]

The pyrolytic conversion of phenyl D-glucopyranosides into 1,6-anhydro-β-D-glucopyranose has been referred to already,[113] and similar treatment of D-galactose was found to give 1,6-anhydro-β-D-galactopyranose, 1,6-anhydro-α-D-galactofuranose, and 1,5-anhydro-α-D-galactofuranose (1,4-anhydro-β-D-galactopyranose).[181] Similar vacuum pyrolyses of pentoses furnished 1,5-anhydrofuranoses (1,4-anhydropentopyranoses) in low (2—5%) yields.[182]

3,4,6-Tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose (Brigl's anhydride) was made to rearrange with dimethyl sulphate in DMF in the presence of barium oxide to give 1,6-anhydro-2,3,4-tri-*O*-methyl-β-D-glucopyranose.[183]

[177] D. R. Hicks, R. Ambrose, and B. Fraser-Reid, *Tetrahedron Letters*, 1973, 2507.
[178] G. O. Aspinall, R. R. King, and Z. Pawlak, *Canad. J. Chem.*, 1973, **51**, 388.
[178a] G. O. Aspinall and R. R. King, *Canad. J. Chem.*, 1973, **51**, 394.
[179] M. J. Robins, R. Mengel, and R. A. Jones, *J. Amer. Chem. Soc.*, 1973, **95**, 4074.
[180] J. W. P. Lin and C. Schuerch, *Macromolecules*, 1972, **5**, 656.
[181] P. Köll, *Chem. Ber.*, 1973, **106**, 3559.
[182] P. Köll, S. Deyhim, and K. Heyns, *Chem. Ber.*, 1973, **106**, 3565.
[183] I. V. Balanina, G. M. Zarubinskii, and S. N. Danilov, *Zhur. obshchei Khim.*, 1973, **43**, 447.

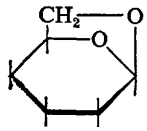
Vinyl ethers of 1,6-anhydro-β-D-glucopyranose have been prepared,[165] and 2-acetamido-3-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-glucopyranose has been prepared for subsequent use in the synthesis of disaccharides (see Scheme 20).[79] Preferential benzylation of 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose gave the diether (26%) and the 3- (5%) and 4-monoethers (21%). The mass spectrum of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose is referred to in Chapter 24.



Reagents: i, NH_3; ii, Ac_2O–py; iii, H_2–Pd

Scheme 20

Reductions of 1,6:2,3- and 1,6:3,4-dianhydrohexoses are mentioned in a previous section (see p. 34), and another related paper has described the synthesis of deoxy-derivatives of 1,6-anhydrohexoses.[184] The trideoxy-analogue (43) has been prepared by three different routes by the same group.[185]



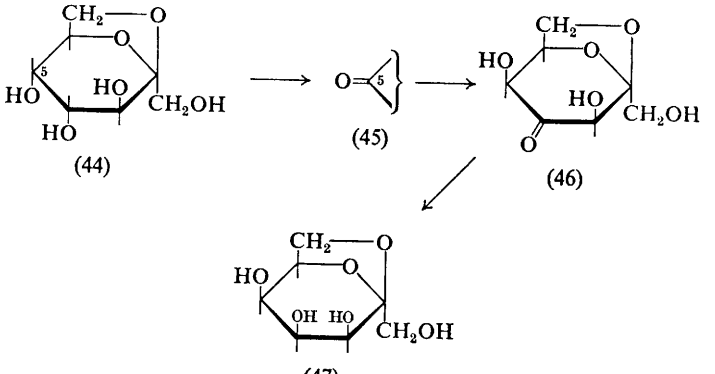
(43)

An improved synthesis of 1,6-anhydrolactose (lactosan) has involved treatment of *o*-chlorophenyl β-D-lactoside hepta-acetate with alkali, and the anhydro-sugar was then utilized in a synthesis of 6-acetamido-6-deoxylactose.[186]

An interesting comparison has been made of the distances between O-2 and O-4 in 1,6-anhydro-β-D-glucopyranose and methyl 3,6-anhydro-α-D-glucopyranoside; these distances have been shown to be 3.30 and 2.76 Å, respectively, by *X*-ray crystallographic measurements.[187] These findings are consistent with measurements made on molecular models and with the finding that the 3,6-anhydride is a stronger acid than the 1,6-anhydride (Schwarz and Totty, unpublished work).

Catalytic oxidation of sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose) (44) gave the ketone (46), presumably by isomerization of the initially formed ketone (45) (Scheme 21); reduction of the ketone (46) gave 2,7-anhydro-β-D-*talo*-heptulopyranose (47).[188]

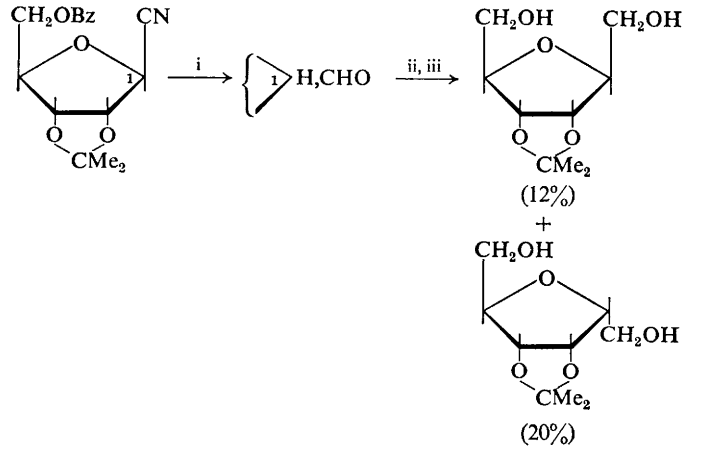
[184] T. Trnka and M. Černý, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3632.
[185] J. Pecka and M. Černý, *Coll. Czech. Chem. Comm.*, 1973, **38**, 132.
[186] S. Tejima and T. Chiba, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 546.
[187] B. Lindberg, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 373.
[188] K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, 1973, **106**, 623.



Scheme 21

A study of the formation of 1,4-anhydropyranoses on treatment of 1-*O*-acetyl-6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-manno- and -talo-pyranoses with azide ion has led to the suggestion that the reactions proceed by generation of the C-1 oxyanions, which then effect the intramolecular displacement at C-4 (*cf.* Vol. 5, p. 51).[189]

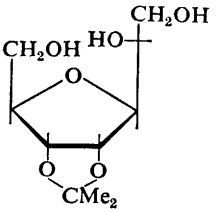
2,5-Anhydrohexose derivatives are reported in Chapter 3 (see *C*-glycosides) and other compounds in this series have been prepared as indicated in Scheme 22.[190] Periodate oxidation of the 2,5-anhydroheptitol (48) has



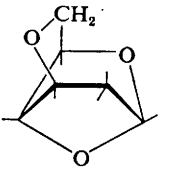
Reagents: i, Ni–NaH_2PO_2–AcOH; ii, $NaBH_4$; iii, NaOMe

Scheme 22

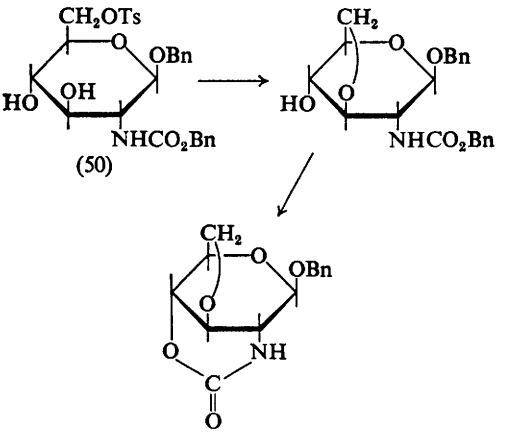
[189] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *J.C.S. Chem. Comm.*, 1973, 142
[190] J. A. Montgomery, K. Hewson, and A. G. Laseter, *Carbohydrate Res.*, 1973, **27**, 303



(48)

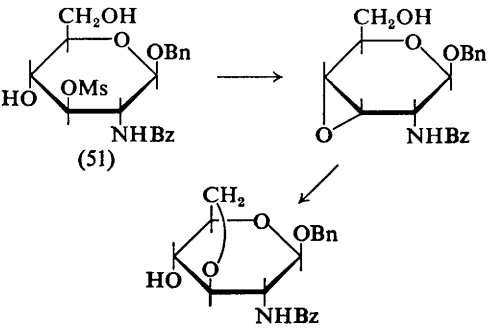


(49)



(50)

Scheme 23



(51)

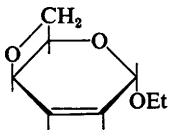
Scheme 24

also provided access to compounds of this type.[191] Derivatives of 1,4-anhydroallitol are dealt with in Chapter 6.

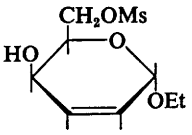
3,6-Anhydro-D-glucal has been found to cyclize spontaneously in acidified chloroform to give the novel 1,4:3,6-dianhydropyranose (49).[192]

An investigation on the formation of 3,6-anhydro-rings in pyranosides containing 2-acylamino-2-deoxy-groups has been reported.[193] The anhydro-ring is suggested to be formed first in the case of the carbobenzyloxy-derivative (50), followed by formation of the 2,4-cyclic carbamate (Scheme 23), whereas with the 3-methanesulphonate (51), it is proposed that the 3,6-anhydro-ring is formed by way of the 3,4-epoxide (Scheme 24).

An unusual 4,6-anhydro-sugar (52) has been obtained by treatment of the 6-methanesulphonate (53) with sodium hydride in ether.[194]



(52)



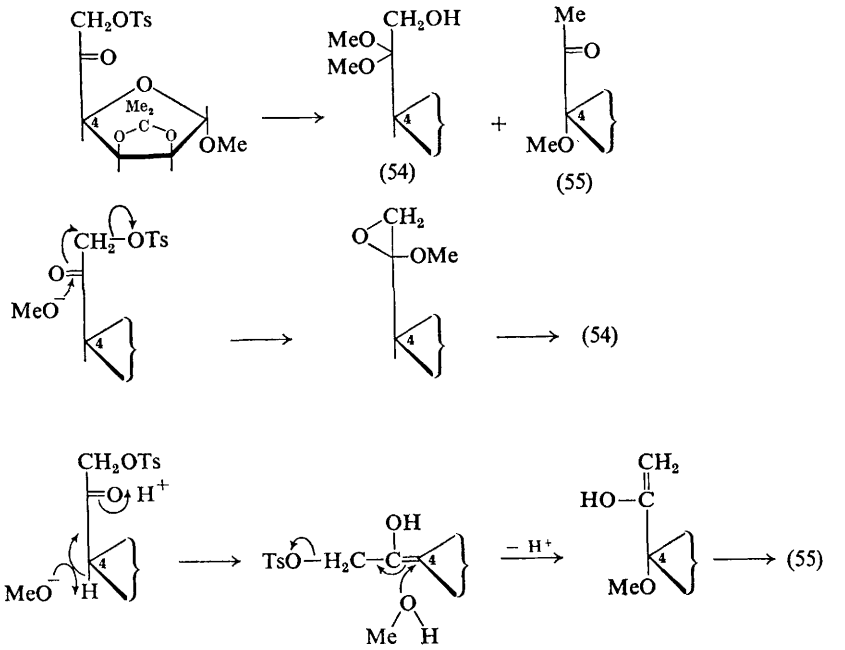
(53)

[191] G. Just and A. Martel, *Tetrahedron Letters*, 1973, 1517.
[192] J. S. Brimacombe, I. Da'aboul, L. C. N. Tucker, N. Calvert, and R. J. Ferrier, *Carbohydrate Res.*, 1973, **27**, 254.
[193] C. A. Johnson and P. H. Gross, *J. Org. Chem.*, 1973, **38**, 2509.
[194] R. J. Ferrier and N. Vethaviyasar, *J.C.S. Perkin I*, 1973, 1791.

5
Acetals

Acetals Derived from Carbohydrate Carbonyl Groups

Treatment of methyl 2,3-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-*lyxo*-hexofuranosid-5-ulose with triethylamine in methanol afforded the acetals (54) and (55) by the pathways suggested in Scheme 25.[195]

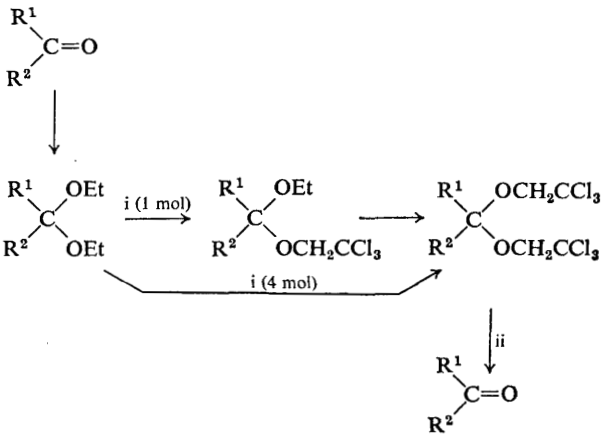


Scheme 25

Non-carbohydrate dialkyl acetals can be converted selectively into mono- and bis-2,2,2-trichloroethyl acetals from which the carbonyl group can be regenerated using activated zinc dust (Scheme 26).[196] It is suggested

[195] A. Dmytraczenko, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1973, **26**, 297.

[196] J. L. Isidor and R. M. Carlson, *J. Org. Chem.*, 1973, **38**, 554.



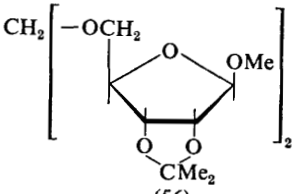
Reagents: i, CCl_3CH_2OH–H^+–PhH; ii, Zn

Scheme 26

that these procedures could be used in carbohydrate chemistry for the protection of carbonyl groups.

Acetals Derived from Carbohydrate Hydroxy-groups

From Single Hydroxy-groups.—Treatment of monohydric alcohols with *N*-bromosuccinimide in DMSO afforded acetals comprising two alcohol residues connected by a methylene bridge [*e.g.* compound (56)].[197]



(56)

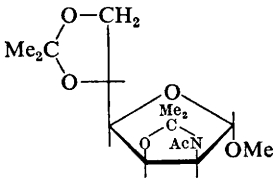
From Diol Groups on Cyclic Carbohydrates.—A review has appeared on oxepin derivatives of sugars,[198] and compounds describable as marginal carbohydrate acetals have been obtained on treatment of glycerol with pyruvic acid or esters thereof and pyruvaldehyde.[199] Reactions of the acetalated lactones so prepared with lithium aluminium hydride and methylmagnesium iodide were also reported.[199a]

Acetonation of L-gulose in the presence of methanol has been shown to furnish methyl 2,3:5,6-di-*O*-isopropylidene-β-L-gulofuranoside.[13]

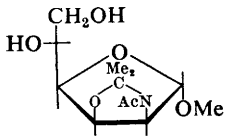
[197] S. Hanessian, P. Lavallee, and A. G. Pernet, *Carbohydrate Res.*, 1973, **26**, 258.
[198] T. R. Hollands, *Chem. Heterocyclic Compounds*, 1972, **26**, 521.
[199] J. Gelas and A. Thiallier, *Carbohydrate Res.*, 1973, **30**, 21.
[199a] P. Calinaud, J. Gelas, and A. Thiallier, *Carbohydrate Res.*, 1973, **30**, 35.

Methyl 4,6-*O*-methylene-D-glycopyranosides having the α-D-*altro*-, α-D-*galacto*-, α- and β-D-*gluco*-, and α-D-*manno*-configurations have been prepared using formaldehyde (prepared from 1,3,5-trioxan) in *p*-dioxan containing boron trifluoride; crystalline methyl 2,3:4,6-di-*O*-methylene-α-D-mannopyranoside was isolated in the course of this work.[200] The ^1H n.m.r. spectra of the 2,3-diacetates of the mono-*O*-methylene derivatives indicated a preference for the $^4C_1$ conformation. Seven-membered acetal rings spanning the 2,3-diol system of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside are referred to in Chapter 16.

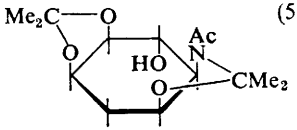
2-Acetamido-2-deoxy-D-glucose, -D-galactose, and -D-mannose yielded 4,6-*O*-isopropylidene derivatives on treatment with 2,2-dimethoxypropane in DMF in the presence of toluene-*p*-sulphonic acid at room temperature.[201] At elevated temperatures, however, the products were more complex, and glycosidic furanoid derivatives were also formed in substantial proportions. In the case of 2-acetamido-2-deoxy-D-mannose, the methyl α-glycofuranoside was formed as both the 2,3:5,6-di-*N*,*O*-isopropylidene (57) and the 5,6-*O*-isopropylidene derivatives; partial hydrolysis of the diacetal with acid gave compound (58).[202] Similar acetalation of *N*-acetyl- and



(57)



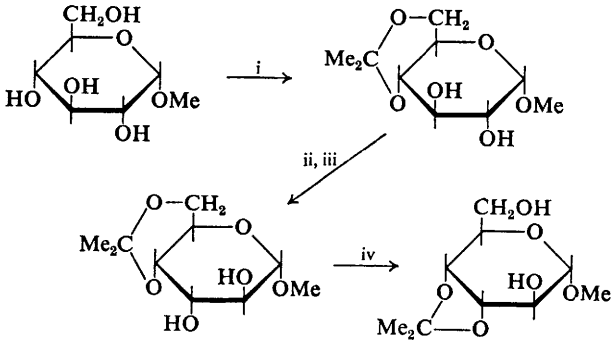
(58)



(59)

N-(benzyloxycarbonyl)-aminocyclitols gave rise to 1,3-dioxolan rings from both *cis*- and *trans*-α-diols.[203] *N*-Acetylamino-groups were also shown to take part in a reaction with 2,2-dimethoxypropane [see compound (59)]. Improved syntheses of 3,4- and 4,6-*O*-isopropylidene derivatives of methyl α-D-altropyranoside are illustrated in Scheme 27.[14] Reaction of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose with either butan-2-one or pentan-3-one in the presence of perchloric acid resulted in preferential exchange of the 4,6-*O*-isopropylidene group, although both groups were exchanged at equilibrium.[204]

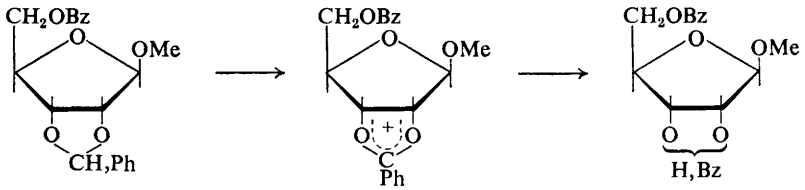
[200] J. C. Goodwin and J. E. Hodge, *Carbohydrate Res.*, 1973, **28**, 213.
[201] A. Hasegawa and H. G. Fletcher, jun., *Carbohydrate Res.*, 1973, **29**, 209.
[202] A. Hasegawa and H. G. Fletcher, jun., *Carbohydrate Res.*, 1973, **29**, 223.
[203] A. Hasegawa and M. Nakajima, *Carbohydrate Res.*, 1973, **29**, 239.
[204] R. S. Glass, S. Kwoh, and E. P. Oliveto, *Carbohydrate Res.*, 1973, **26**, 181.



Reagents: i, $Me_2C(OMe)_2$–TsOH–DMF; ii, MsCl–py; iii, NaOH; iv, H^+–Me_2CO

Scheme 27

Further studies on the preparation of *O*-benzylidene acetals have indicated that benzal bromide in pyridine is a satisfactory reagent for use in the presence of trityl and acetyl groups; a number of carbohydrate acetals were described.[205] Trityl fluoroborate (or trityl chloride in the presence of stannic chloride) has been used to convert five-membered benzylidene acetals into the corresponding benzoxonium ions, which gave a mixture of isomeric benzoates on hydrolysis (Scheme 28).[206] With



Scheme 28

related acetals of pyrimidine nucleosides, anhydro-derivatives were readily obtained (see Chapter 21). ^1H N.m.r. and c.d. spectroscopy have been used to study the stereochemistry at the acetal carbon atoms of nucleoside 2′,3′-*O*-benzylidene derivatives.[207] The use of benzaldehyde dimethyl acetal has been recommended for the preparation of the 4,6-*O*-benzylidene derivative of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside.[208] The mass spectra of methyl 4,6-*O*-benzylidenehexopyranosides and various derivatives thereof are referred to in Chapter 24.

Condensation of 2-furaldehyde with D-xylose and D-glucose afforded the 3,5- and 4,6-*O*-furylidene derivatives, respectively, of these sugars.[209]

[205] P. J. Garegg and C.-G. Swahn, *Acta Chem. Scand.*, 1972, **26**, 3895.
[206] S. Hanessian and A. P. A. Staub, *Tetrahedron Letters*, 1973, 3551.
[207] A. M. Belikova, N. I. Grineva, and G. N. Kabashea, *Tetrahedron*, 1973, **29**, 2277.
[208] K. Yamamoto and T. Hayashi, *Bull. Chem. Soc. Japan*, 1973, **46**, 656.
[209] M. Košík, V. Demianová, V. Kováčik, and P. Kováč, *Chem. and Ind.*, 1973, 94.

From these observations, it is concluded that such acetals may be formed from free sugars in processes (such as pulp-cooking) in which 2-furaldehyde is formed under acidic conditions.

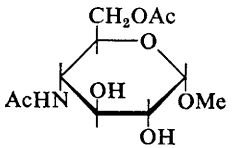
From Diol Groups on Acyclic Carbohydrates.—Treatment of 1,6-di-*O*-benzoyl-D-mannitol with *N*-bromosuccinimide in DMSO and carbon tetrachloride gave the 2,3:4,5-di-*O*-methylene derivative in good yield.[197] Di-*O*-benzylideneallitol has been shown to have the 2,4:3,5-structure (see Chapter 6), and D-gluconamide afforded the 3,4:5,6-di-*O*-isopropylidene derivative on acetonation (see Chapter 22).

A detailed study of the sulphonylation of 2,4-*O*-methylenexylitol is referred to in Chapter 27.

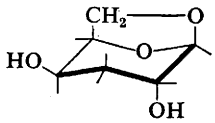
6
Esters

Carboxylic Esters

Partial acetylation of the 4-acetamido-4-deoxy-D-glucose derivative (60) using either acetic anhydride or acetyl chloride in pyridine gave, in addition to the 2,3-diacetate, the 2- and 3-acetates in the ratios 76 : 24 and 58 : 42, respectively.[210] Partial acetylation of methyl α-D-glucopyranoside has been found to give the following products: 2,3,4,6-tetra-acetate (20), 2,3,6-triacetate (35), 2,6- (410), 2,3- (385), and 2,4-diacetates (490), and 6- (820), 3- (390), 2-acetates (275 parts).[211] Selective benzoylation of 1,6-anhydro-3-deoxy-β-D-*xylo*-hexopyranose (61) has yielded the axial 2-ester, presumably as a result of hydrogen-bonding of the hydroxy-group at C-2 with O-5.[212]



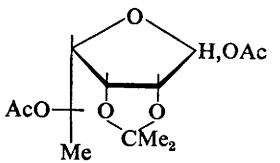
(60)



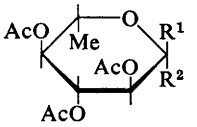
(61)

Acetolyses of 2,3-*O*-isopropylidene-L-rhamnofuranose or its 1,5-diacetate have yielded a mixture of acetates that afforded L-rhamnose (45%) and L-quinovose (55%) after deacetylation; acetolysis of L-rhamnopyranose derivatives did not result in epimerization at C-2.[15] In a related paper, acetolysis of (62) was shown to give the L-quinovose tetra-acetates (63) and (64) following deacetylation of the products of acetolysis and reacetylation. It is not surprising, therefore, that 6-deoxy-L-glucofuranosyl nucleosides were formed, *inter alia*, when the crude products from acetolysis of (62) were coupled with purines.[213] Careful characterization of the products of acetolysis is clearly necessary in situations analogous to those indicated, and it would appear that aldofuranoses with *cis*-related hydroxy-groups at C-2 and C-3 are susceptible to partial epimerization at C-2 during acetolysis.

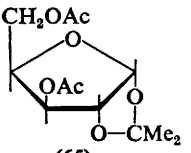
[210] K. Čapek and J. Jarý, *Coll. Czech. Chem. Comm.*, 1973, **38**, 2518.
[211] F. Bottino, M. Santagati, and M. Piatelli, *Ann. Chim. (Italy)*, 1972, **62**, 782.
[212] J. M. Macleod, L. R. Schroeder, and P. A. Seib, *Carbohydrate Res.*, 1973, **30**, 337.
[213] L. M. Lerner, *J. Org. Chem.*, 1972, **37**, 4386.



(62)

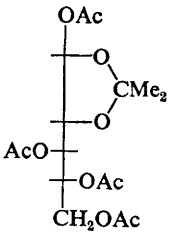


(63) R^1 = OAc, R^2 = H
(64) R^1 = H, R^2 = OAc

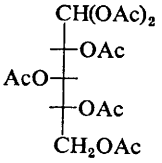


(65)

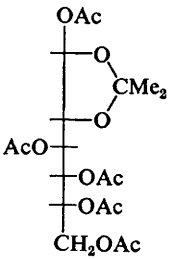
Acetolysis of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (65), using acetic anhydride and sulphuric acid, gave the acyclic derivative (66), which was transformed into the hexa-*O*-acetyl-*aldehydo*-D-xylose aldehydrol (67) on prolonged reaction.[214] In the presence of acetic acid, D-xylofuranose tetra-acetates were the main products of acetolysis and only a small proportion of (67) was obtained. However, to prepare pure D-xylofuranose



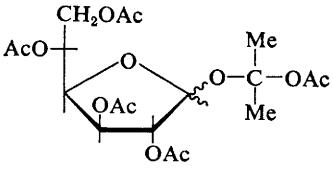
(66)



(67)



(68)

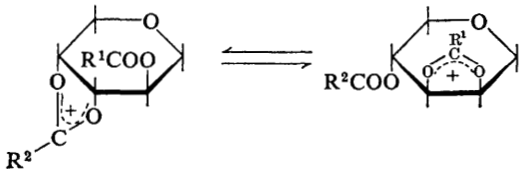


(69)

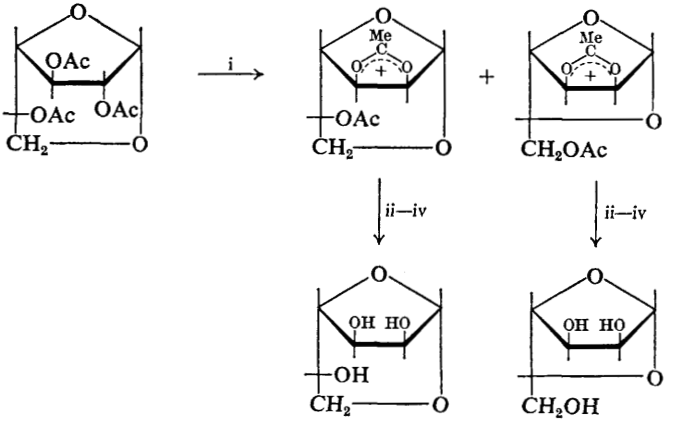
tetra-acetates, it was found best to deacetonate (65) and then to acetylate the resulting 3,5-di-*O*-acetyl-D-xylofuranose. Acetolysis of 1,2-*O*-isopropylidene-α-D-glucofuranose or its 3,5,6-triacetate has been shown (^{1}H n.m.r. evidence) to furnish the acyclic *aldehydo*-D-glucose derivative (68) and not the alternative structure (69) suggested.

Pedersen's group has continued its studies on the rearrangements of acyloxonium ions by examining the system shown in Scheme 29; the relative stabilities of the acyloxonium ions were found to be highly dependent on the nature of the R groups.[215] Paulsen's group has also carried out further work in this area by studying the system shown in Scheme 30.[216]

[214] A. Magnani and Y. Mikuriya, *Carbohydrate Res.*, 1973, **28**, 158.
[215] S. Jacobsen, I. Lundt, and C. Pedersen, *Acta Chem. Scand.*, 1973, **27**, 453.
[216] P. Durette, P. Köll, H. Meyborg, and H. Paulsen, *Chem. Ber.*, 1973, **106**, 2333.



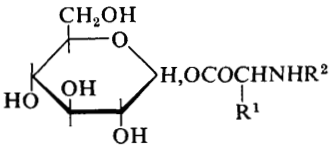
Scheme 29



Reagents: i, CF_3SO_3H; ii, H_2O; iii, Ac_2O–py; iv, MeO^-–MeOH

Scheme 30

The chloroacetyl group has been evaluated as a protecting group for the primary hydroxy-group of D-glucose with a view to assessing its use in polymer-support syntheses of oligosaccharides.[217] The preparation and properties of fatty-acid esters of sucrose have been reviewed.[217a] A number of 1-*O*-acylaminoacyl esters of α- and β-D-glucopyranose [*e.g.* (70)] have been prepared by way of the 2,3,4,6-tetra-*O*-benzyl ether; it was found that



(70)

1,2-acyl migration in α-anomers occurs more readily when R^2 = $COCMe_3$ than when R^2 = Ac.[218]

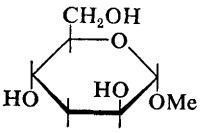
[217] D. Y. Gagnaire and P. J. A. Vottero, *Carbohydrate Res.*, 1973, **28**, 165.
[217a] J. Broniarz and J. Szymanowski, *Wiadomosci. Chemi.*, 1973, **27**, 327.
[218] D. Keglević, Š. Valenteković, G. Roglić, D. Goleš, and F. Plavšić, *Carbohydrate Res.*, 1973, **29**, 25.

1,6-Di-*O*-(3-nitropropanoyl)-β-D-glucopyranose has been extracted from *Astragalus cibarius*.[219]

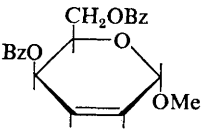
Methyl α-D-galactopyranoside has been benzoylated, and the crude benzoate was saponified to give the 6-ester (30%).[220] Treatment of lactose with benzoyl chloride and aqueous sodium hydroxide afforded 1,2,6,2′,3′,-4′,6′-hepta-*O*-benzoyl-β-lactose, which was debenzoylated with methanolic ammonia to give the free sugar and 6-*O*-benzoyl-lactose; the latter result parallels those obtained with other disaccharides, and reasons for the stability of the 6-ester were discussed.[221]

Hydrolyses of variously substituted β-D-glucopyranosyl benzoates have been studied in detail over a range of pH values.[222] Under acid conditions, the hydrolyses were shown to be analogous to those of glycosides, proceeding by *A*-1 processes. At higher pH values, complex kinetics were observed and benzoyl migration competed with hydrolysis when the hydroxy-groups at either C-2 or C-6 were unsubstituted. No migration occurred during hydrazinolysis and simple kinetics were followed.

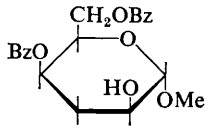
Treatment of 2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosylamine with ammonia afforded *N*-benzoyl-β-D-mannopyranosylamine, showing that O → N acyl migration is stereospecific in *O*-acylated glycosylamines and takes place when the groups at C-1 and C-2 are *cis*-related.[223] Studies of the reactions of sugar esters with ammonia have been extended to nicotinoates[224] and the migration of pivaloyl esters of nucleosides has been examined.[225]



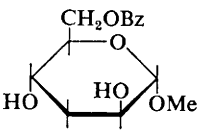
(71)



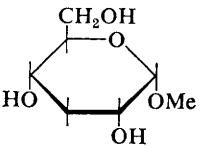
(72)



(73)



(74)



(75)

[219] F. R. Stermitz and W. T. Lowry, *Phytochemistry*, 1972, **11**, 3525.
[220] D. H. Hollenberg, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1973, **28**, 135.
[221] I. M. Vazquez, I. M. E. Thiel, and J. O. Deferrari, *Carbohydrate Res.*, 1973, **26**, 351.
[222] A. Brown and T. C. Bruice, *J. Amer. Chem. Soc.*, 1973, **95**, 1593.
[223] J. F. Sproviero, *Carbohydrate Res.*, 1973, **26**, 357.
[224] E. A. Forlano, J. O. Deferrari, and R. A. Cadenas, *Anales Asoc. quím. argentina*, 1972, **60**, 323 (*Chem. Abs.*, 1973, **78**, 4457*n*).
[225] J. Baker, M. Jarman, and J. A. Stock, *J.C.S. Perkin I*, 1973, 665.

An attempt has been made to effect configurational inversions on hydroxy-groups in carbohydrates using benzoic acid as the nucleophile (*cf.* Vol. 6, p. 61). Thus, treatment of the triol (71) with benzoic acid, triphenylphosphine, and diethyl azodicarboxylate in THF gave (72), (73), and (74), in yields of 23, 47, and 12%, respectively.[226] Displacement at C-6 is therefore indicated to be achieved more easily than at C-4, but no displacement of the hydroxy-group at C-2 is observed. A hex-3-enopyranoside was the main product derived from similar treatment of the isomeric triol (75).

The *o*-nitrobenzoate group has been recommended as a hydroxy-protecting group for alcohols, since it can be removed in high yield by reduction with zinc dust and ammonium chloride.[227]

Orthoesters

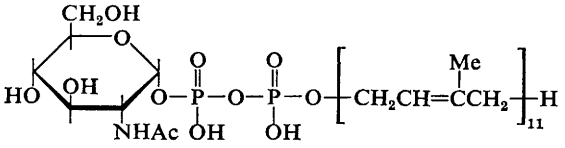
The use of monosaccharide orthoesters in the synthesis of linear polysaccharides has been reviewed.[48] Mono- and oligo-saccharide 1,2-orthoesters with complex alcohols have been obtained in high yield by reaction of acylated 1,2-*cis*-glycosyl halides with partially protected sugar derivatives in the presence of silver nitrate and 2,4,6-trimethylpyridine in dry acetonitrile.[228] The reaction was shown to proceed by way of an acylated 1,2-*trans*-glycosyl nitrate.

Orthoesters have been shown to be formed following peroxy-acid oxidation of certain unsaturated sugars [178, 178a] (see p. 110).

Phosphates

Syntheses of D-fructose 1,2-cyclic phosphate and 2-phosphate have been described and the hydrolysis of these esters with acid was investigated;[229] syntheses of β-L-fucopyranosyl and β-L-rhamnopyranosyl phosphates have also been described.[230]

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosylammonium phosphate has been prepared by the action of crystalline phosphoric acid on 2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, and was converted into the 'lipid intermediate' P^1-(2-acetamido-2-deoxy-D-glucopyranosyl)



(76)

[226] G. Alfredsson and P. G. Garegg, *Acta Chem. Scand.*, 1973, **27**, 724.
[227] D. H. R. Barton, I. H. Coates, and P. G. Sammes, *J.C.S. Perkin I*, 1973, 599.
[228] S. E. Zurabyan, M. M. Tikhomirov, V. A. Nesmeyanov, and A. Y. Khorlin, *Carbohydrate Res.*, 1973, **26**, 117.
[229] H. Taniguchi and M. Nakamura, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 2373.
[230] H. S. Prihar and E. J. Behrman, *Biochemistry*, 1973, **12**, 997.

P^2-ficaprenyl pyrophosphate (76).[231] Syntheses of α-D-mannopyranosyl phosphate esters of farnesol, ficaprenol, dolichol, and citronellol have been carried out using standard reactions.[232]

Phosphorylation of D-glucose derivatives by mono- and di-methyl phosphite and by phosphorous acid gave the 6-*O*-phosphites with methyl α-D-glucopyranoside and 1,2-*O*-cyclohexylidene-α-D-glucofuranose.[233] The reaction of 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-4-*S*-thiobenzoyl-α-D-glucopyranose with phosphoric acid furnished 4-thio-α-D-glucopyranosyl phosphate.[234] The McDonald procedure (*Carbohydrate Res.*, 1966, **3**, 117) has been applied to the phosphorylation of deoxy-D-glucoses and disaccharides; 6-, 4-, 3-, and 2-deoxy-D-glucosyl phosphates were prepared from the corresponding β-acetates using this method.[235, 235a] The α- and β-anomers of 4-*O*-methanesulphonyl-D-galactopyranosyl phosphate have been prepared by reaction of the benzoylated glycosyl acetates with phosphoric acid.[236] Appropriate disaccharide phosphates were similarly obtained from the β-octa-acetates of maltose and cellobiose.[237]

A detailed study has been made of the g.l.c. and mass spectrometry of the TMS-derivatives of phosphate esters of sugars [238] (see also Chapter 27).

The interconversion of the 6-phosphates of D-glucose, D-mannose, and D-fructose has been found to be catalysed by bi- and ter-valent metal ions, by virtue of their Lewis-acid character.[239]

A convenient, large-scale preparation of D-arabinose 5-phosphate has been achieved from 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose.[240] The 5′- and 6′-phosphates of 9-α-D-mannofuranosyladenine have been prepared by phosphorylation of the 2′,3′-*O*-isopropylidenated nucleoside with phosphorus oxychloride and triethyl phosphate; the 5′-phosphate was the only product formed when the unprotected nucleoside was used.[241]

A review of the chemistry and biochemistry of sugar nucleoside diphosphates has been published.[242] Analogues of UDP-D-glucose containing a

[231] C. D. Warren, Y. Konami, and R. W. Jeanloz, *Carbohydrate Res.*, 1973, **30**, 257.
[232] C. D. Warren and R. W. Jeanloz, *Biochemistry*, 1973, **12**, 5031, 5038.
[233] E. E. Nifantev, I. P. Andrianova, N. P. Kostromin, and Chan Din Dat, *Zhur. obshchei Khim.*, 1973, **43**, 1619.
[234] N. K. Kochetkov, V. N. Shibaev, Yu. Yu. Kusov, and M. F. Troitsky, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 425.
[235] V. N. Shibaev, Yu. Yu. Kusov, Sh. Kuchar, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 430.
[235a] V. N. Shibaev, Yu. Yu. Kusov, Sh. Kuchar, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 922.
[236] V. N. Shibaev, Yu. Yu. Kusov, M. F. Troitsky, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 1862.
[237] V. N. Shibaev, Yu. Yu. Kusov, M. F. Troitsky, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 182.
[238] D. J. Harvey and M. G. Horning, *J. Chromatog.*, 1973, **76**, 51.
[239] B. E. Tilley, D. W. Porter, and R. W. Gracy, *Carbohydrate Res.*, 1973, **27**, 289.
[240] J. Stverteczky, P. Szabo, and L. Szabo, *J.C.S. Perkin I*, 1973, 872.
[241] M. J. Taylor, B. D. Kohn, W. G. Taylor, and P. Kohn, *Carbohydrate Res.*, 1973, **30**, 133.
[242] N. K. Kochetkov and V. N. Shibaev, *Adv. Carbohydrate Chem. Biochem.*, 1973, **28**, 307.

variety of substituents (OH, NH_2, OMe, Cl, Br, I) at C-5 of the heterocyclic moiety have been synthesized.[243]

^{13}C N.m.r. spectroscopy has been used to examine the anomeric composition of equilibrated solutions of D-fructofuranose 6-phosphate and 1,6-diphosphate; the anomeric ($\alpha : \beta$) ratios obtained were 19 : 81 and 23 : 77, respectively.[244]

Inversion of the configuration at phosphorus occurred when methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (*S*)-4,6-phosphochloridate was treated with such nucleophiles as ethanol and dimethylamine whereas the reaction with methylmagnesium bromide furnished a product of retained configuration.[244a]

A number of sugar phosphonates are described in Chapter 14, and other nucleoside phosphates are reported in Chapter 21.

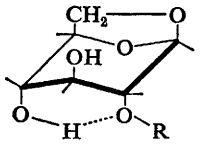
Sulphonates

Selective sulphonylation of methyl α-D-xylopyranoside gave mainly the 2,4-disulphonate with two equivalents of methanesulphonyl chloride in pyridine, and the 2-sulphonate was obtained when one equivalent of the reagent was employed.[245] The order of reactivity for the α-anomer is thus O-2 > O-4 > O-3, whereas for the β-anomer the order is O-4 > O-3 > O-2. Similar results were obtained on sulphonation of benzyl α-D-xylopyranoside with toluene-*p*-sulphonyl chloride in pyridine. [The results for the α-anomers do not accord with those previously reported (see Vol. 5, p. 47), but are more in keeping with other results on similar systems.] Dimolar methanesulphonylation of benzyl α-D-glucopyranoside yielded principally the 2,6-disulphonate.

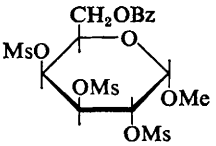
Selective toluene-*p*-sulphonylation of the 1,6-anhydro-sugar (61) gave the 2-sulphonate, whereas the 2-*O*-methyl and 2-*O*-benzyl ethers of 1,6-anhydro-β-D-glucopyranose (77) afforded the 4-sulphonates.[212] 6′-*O*-Toluene-*p*-sulphonylmaltose has been obtained by enzymic hydrolysis of mono-*O*-toluene-*p*-sulphonylcyclohexa-amylose.[246]

Displacement of the methanesulphonyloxy-groups of 1′,4,6′-tri-*O*-methanesulphonylsucrose penta-acetate with either azide or benzoate ions occurred in the order 4 ≈ 6′ > 1′ to give a mixture of 1′,4,6′-trisubstituted and 4,6′-disubstituted products.[247] It was also shown that the 6-sulphonyloxy-group of 6,6′-di-*O*-toluene-*p*-sulphonylsucrose hexa-acetate is the more reactive towards nucleophilic displacement.[248] The 4-*O*-methanesulphonyloxy-group in the D-galactoside 2,3,4-trimethanesulphonate (78) has been shown to be the most easily displaced.[220]

[243] V. N. Shibaev, G. P. Eliseeva, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2095.
[244] T. A. W. Koerner, L. W. Cary, N. C. Bhacca, and E. S. Younathan, *Biochem. Biophys. Res. Comm.*, 1973, **51**, 543.
[244a] T. D. Inch and G. J. Lewis, *Tetrahedron Letters*, 1973, 2187.
[245] R. C. Chalk and D. H. Ball, *Carbohydrate Res.*, 1973, **28**, 313.
[246] L. D. Melton and K. N. Slessor, *Canad. J. Chem.*, 1973, **51**, 327.
[247] L. Hough and K. S. Mufti, *Carbohydrate Res.*, 1973, **29**, 291.
[248] L. Hough and K. S. Mufti, *Carbohydrate Res.*, 1972, **25**, 497.

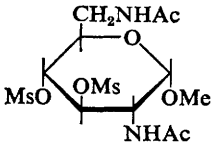


(77) R = Me or Bn

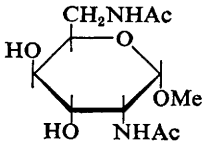


(78)

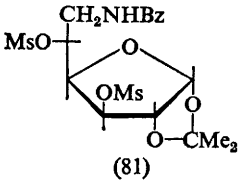
Attempts to deacetylate 2,3,4,5-tetra-*O*-acetyl-1,6-di-*O*-toluene-*p*-sulphonylallitol and the 1,6-di-*O*-methanesulphonate, using hydrogen chloride in hot methanol, afforded the corresponding 6-substituted derivatives of 1,4-anhydro-DL-allitol.[249] Both the 3- and 4-methanesulphonyloxy-groups were solvolysed, presumably with acetamido-group participation, when the D-glucopyranoside dimethanesulphonate (79) was heated in wet 2-methoxymethanol; the product of solvolysis is methyl 2,6-diacetamido-2,6-dideoxy-α-D-gulopyranoside (80).[250]



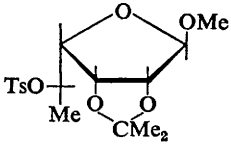
(79)



(80)



(81)



(82)

Contrary to earlier reports, the 6-benzamido-group has been demonstrated to participate in the displacement of the 5-methanesulphonyloxy-group from (81).[251] Nucleophilic displacements on methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-L-rhamnofuranoside (82) have been found to yield, *inter alia*, the two possible unsaturated sugars, even in reactions with such a highly nucleophilic, but weakly basic, species as azide ion.[252]

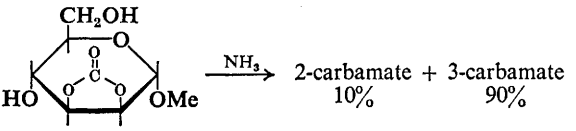
Chapters 4 and 7 contain references to the use of sulphonates in the formation of anhydro- and halogeno-sugars, respectively.

[249] J. M. Ballard and B. E. Stacey, *Carbohydrate Res.*, 1973, **30**, 91.
[250] J. S. Brimacombe, I. Da'aboul, and L. C. N. Tucker, *Carbohydrate Res.*, 1972, **25**, 522.
[251] M. Miljković, T. Satoh, M. Konopka, E. A. Davidson, and D. Miljković, *J. Org. Chem.*, 1973, **38**, 716.
[252] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *Carbohydrate Res.*, 1973, **31**, 146.

Other Esters

The 3-*O*- and 2,3-di-*O*-sulphopropyl esters of D-glucose have been prepared.[253]

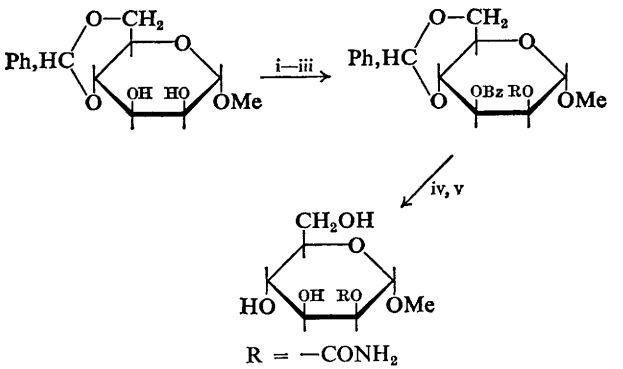
N-Phenylcarbamoyl and *N*-methyl-*N*-phenylcarbamoyl groups have been examined with a view to their use as 'persistent' blocking groups that can also participate in glycoside-forming reactions.[254] Thus, 6-*O*-acetyl-2,3,4-tri-*O*-(*N*-phenylcarbamoyl)-α-D-glucopyranosyl bromide reacted stereospecifically with methanol to give the β-glycoside, whereas the analogous *N*-methyl-*N*-phenyl derivative furnished a mixture of α- and β-glycosides in the ratio of 3 : 7. Methyl 2- and 3-*O*-carbamoyl-α-D-mannopyranosides have been synthesized by way of ammonolysis of methyl α-D-mannopyranoside 2,3-cyclic carbonate (Scheme 31); the 3- and 2-carbamates



Scheme 31

were equilibrated in ethanolic triethylamine to give a mixture containing these esters in the ratio of *ca.* 3 : 1.[255] A more efficient synthesis of the 2-carbamate is shown in Scheme 32.

The reaction of a 3 : 1 mixture of 2,3,6-tri-*O*-methyl-α- and -β-D-glucopyranoses with phenyl isocyanate has been examined in a variety of



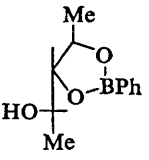
Reagents: i, BzCl–py; ii, *p*-$NO_2C_6H_4OCOCl$; iii, liq. NH_3; iv, H_2–Pd–C; v, NaOMe–MeOH

Scheme 32

[253] A. I. Usov, Z. I. Kuznetsova, and V. S. Arkhipova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 1377.
[254] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1973, **27**, 63.
[255] S. Omoto, T. Takita, K. Maeda, and S. Umezawa, *Carbohydrate Res.*, 1973, **30**, 239.

solvents (acetone, benzene, DMSO, THF, *etc.*) containing triethylamine.[256] The anomer ratio of the product mixture was found to be solvent-dependent; the ratio of β- : α-anomers varied from 4.55 in benzene to 0.49 in DMSO, and it was proposed that mutarotation accompanies carbanilation at the anomeric centre *via* a solvated complex.

The reaction of a number of representative triols with benzeneboronic anhydride has been examined, but only *xylo*-pentane-2,3,4-triol gave a single product (83).[257] All the other products were mixtures of structural

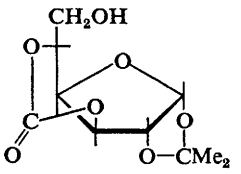


(83)

isomers and, generally, it was found that six-membered rings are preferred if they do not contain an axial substituent, otherwise five-membered rings are formed. The reactions of benzeneboronic acid and its 4-methoxy- and 3-nitro-derivatives with D-glucose, D-mannose, and D-fructose at various pH values have been examined by polarimetry,[258] and the 1,2:3,5-bis(benzeneboronate) of α-D-glucofuranose has been used in a convenient synthesis of 6-*O*-methyl-D-glucose.[152] The mass spectra of benzeneboronates derived from methyl glycosides have been investigated [259] (see Chapter 24), and ^{11}B n.m.r. spectroscopy has been used to demonstrate the existence of boron-containing complexes in solutions of sugars containing borax or benzeneboronic acid [260] (see also Chapter 23).

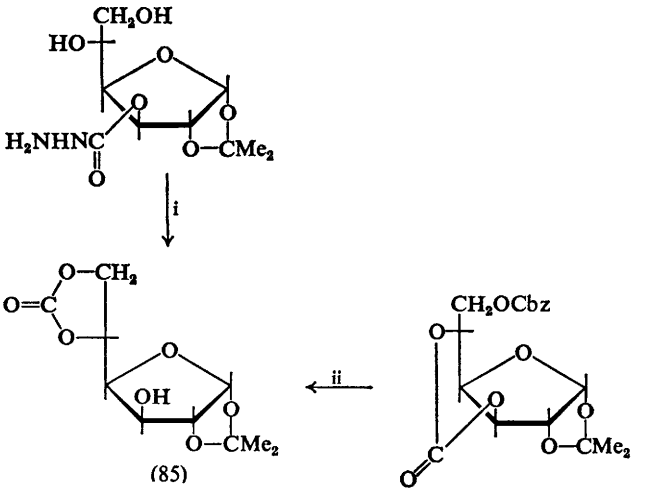
The reaction of 2′,3′-*O*-isopropylideneuridine with an excess of thionyl chloride afforded bis[1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil] 5′-sulphite in moderate yield instead of the expected 5′-chloro-derivative.[261]

The instability of six-membered cyclic carbonates has been noted, and attempts to prepare the 3,5-cyclic carbonate (84) yielded instead the



(84)

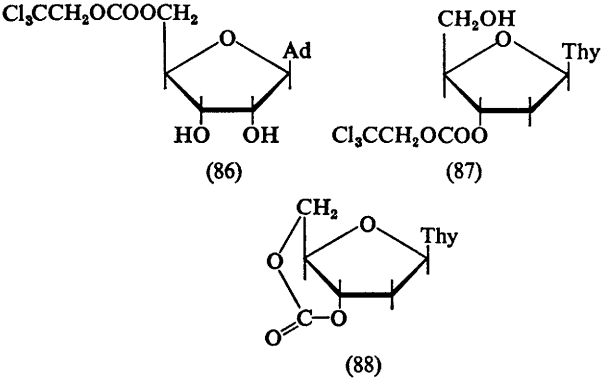
[256] Y.-H. Yeh and R. D. Gilbert, *Carbohydrate Res.*, 1973, **30**, 155.
[257] I. R. McKinley and H. Weigel, *Carbohydrate Res.*, 1973, **31**, 17.
[258] S. A. Barker, A. K. Chopra, B. W. Hatt, and P. J. Somers, *Carbohydrate Res.*, 1973, **26**, 33.
[259] D. S. Robinson, J. Eagles, and R. Self, *Carbohydrate Res.*, 1973, **26**, 204.
[260] G. R. Kennedy and M. J. How, *Carbohydrate Res.*, 1973, **28**, 13.
[261] P. C. Srivastava and R. J. Rousseau, *Carbohydrate Res.*, 1973, **27**, 455.



Reagents: i, Br_2–py; ii, H_2–Pt

Scheme 33

5,6-cyclic carbonate (85) (Scheme 33).[262] In both cases, it was assumed that the 3,5-cyclic carbonate is formed initially, but that it subsequently rearranges by way of the 3,5,6-orthocarbonate. Attempts to prepare nucleoside and deoxynucleoside 3′,5′-cyclic carbonates have also been described.[263] Treatment of 5′-*O*-(2,2,2-trichloroethoxycarbonyl)adenosine (86) with base gave only adenosine 2′,3′-cyclic carbonate, presumably by way of an intermediate 2′,5′- or 3′,5′-dinucleoside carbonate. Whereas the 2′-deoxy-analogue of (86) did not afford the 3′,5′-cyclic carbonate on treatment with base, cyclization to give the six-membered cyclic carbonate (88)



[262] G. P. Rizzi, *J. Org. Chem.*, 1973, **38**, 618.
[263] J. R. Tittensor and P. Mellish, *Carbohydrate Res.*, 1972, **25**, 531.

was achieved with 3′-*O*-(2,2,2-trichloroethoxycarbonyl)thymidine (87). Decomposition of the cyclic carbonate (88) occurred readily in ethanol to give equal proportions of 5′- and 3′-*O*-ethoxycarbonylthymidines.

Selective sulphation of the *axial* hydroxy-group in methyl 2-acetamido-6-*O*-acetyl-2-deoxy-α-D-galactopyranoside has been found to occur with sulphur trioxide–pyridine, whereas both hydroxy-groups were esterified with methanesulphonyl chloride–pyridine.[264] Sucrose penta- and hexa-benzoates reacted with sulphuryl chloride in pyridine at −75 °C to provide syntheses of penta-*O*-benzoylsucrose 1′,6,6′-tris(chlorosulphate) and hexa-*O*-benzoylsucrose 6,6′-bis(chlorosulphate).[265] The 6- and 6′-chlorosulphate groups in these esters were readily converted into chloro-groups under a variety of conditions, but the 1′-chlorosulphate group was best displaced with sodium chloride in HMPA.

[264] S. Hirano, *Carbohydrate Res.*, 1973, **27**, 265.
[265] R. Khan, *Carbohydrate Res.*, 1972, **25**, 504.

7
Halogenated Sugars

Glycosyl Halides

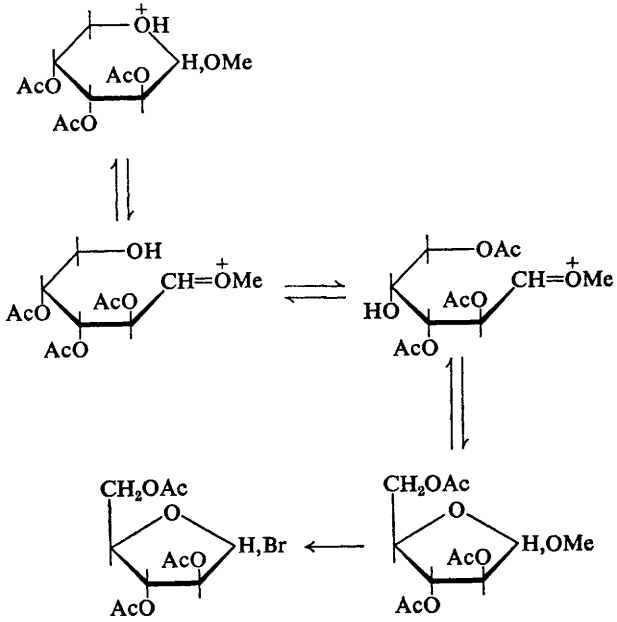
A number of examples of the use of glycosyl halides in the synthesis of glycosides are referred to in Chapter 3.

Following their papers published in 1968 on the addition of nitrosyl chloride to glycals and on the chemistry of the dimeric products that are formed, Lemieux and his colleagues have published a series of eight papers in which further aspects of the chemistry of these glycosyl chlorides – particularly in the synthesis of α-glycosides (*cf.* Chapter 3) – are described.[266, 267]

Hydrolysis and halogen-exchange reactions of 1,2-*trans*-*O*-acetyl-D-glucosyl chlorides have been studied in acetone; halogen exchange was indicated to proceed *via* S_N2 processes, whereas hydrolysis followed an S_N1 pathway.[268] Methanolysis of 6-*O*-acetyl-2,3,4-tri-*O*-(*N*-phenylcarbamoyl)-α-D-glucopyranosyl bromide afforded the β-glycoside, as a consequence of neighbouring-group participation by the C-2 ester, whereas the analogous 2-(*N*-methyl-*N*-phenylcarbamate) gave both α- and β-glycosides.[254]

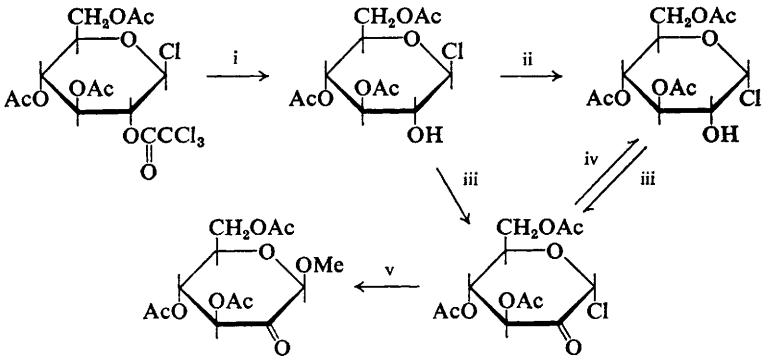
Ring-contraction was found to occur when methyl 2,3,4-tri-*O*-acetyl-β-D-arabinopyranoside was treated with hydrogen bromide, and both acetylated arabino-furanosyl and -pyranosyl bromides were formed.[269] Ring-contraction was not observed when either methyl 2,3,4-tri-*O*-benzoyl-β-D-arabinopyranoside or the corresponding 3-*O*-acetyl-2,4-dibenzoate were treated similarly, but the 4-*O*-acetyl-2,3-dibenzoate did undergo a ring-contraction, which was ascribed to the greater aptitude of the acetyl group to migrate (Scheme 34). Other pentoside esters were shown to behave similarly. The same workers have shown that acylated pentoses or methyl pentosides on treatment with dibromomethyl methyl ether and zinc bromide are rapidly converted into the acylated pentopyranosyl bromides, which are slowly transformed into 2-bromo-2-deoxypentopyranosyl bromides.[270] The formation and opening of intermediate acyloxonium ions accounted for these results.

[266] R. U. Lemieux, T. L. Nagabhushan, and K. James, *Canad. J. Chem.*, 1973, **51**, 1.
[267] R. U. Lemieux, Y. Ito, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 7.
[268] G. Pass, G. O. Phillips, and A. Samee, *J.C.S. Perkin II*, 1973, 932.
[269] K. Bock and C. Pedersen, *Carbohydrate Res.*, 1973, **29**, 331.
[270] K. Bock, C. Pedersen, and P. Rasmussen, *J.C.S. Perkin I*, 1973, 1456.



Scheme 34

A novel class of compounds to have received attention is the glycopyranosylulose chlorides, and preparations of the acetylated α-D-*arabino*-hexosyl, α-D-*lyxo*-hexosyl, and β-L-*erythro*-pentosyl compounds have been reported (Scheme 35).[271] Methanolyses of these compounds proceeded more slowly than those of the corresponding 2-hydroxyglycosyl chlorides.



Reagents: i, NH_3; ii, $TiCl_4$; iii, RuO_4; iv, $LiAlH_4$; v, MeOH

Scheme 35

[271] P. M. Collins, W. G. Overend, and B. A. Rayner, *Carbohydrate Res.*, 1973, **31**, 1.

Glycosyl chlorides of methyl D-mannuronate have been described,[271a] and the bromide from benzyl (2,3,4-tri-*O*-benzyl-D-glucuronate) has been used to prepare the corresponding glycosyl esters and aryl glycosides.[272]

4-Azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl chloride and the isomeric 6-azido-compound have been prepared,[273] and it has been shown that α-D-glucopyranosyl fluoride can be used in glycosyl-transfer reactions catalysed by amylosucrase to form either sucrose or an amylopolysaccharide.[274]

Glycosyl bromides have been obtained on free-radical bromination of benzyl glycosides.[163]

Other Halogenated Derivatives

A considerable number of papers have discussed this class of compound, which will now be considered in order of increasing atomic weight of the halogen.

A review (in Czech) on the synthesis and biological properties of deoxyfluoro-sugars has appeared,[275] and the number of specifically substituted deoxyfluoro-sugars reported has continued to increase. 2,6-Dideoxy-2-fluoro-D-glucose has been prepared from 1,6-anhydro-2-deoxy-2-fluoro-β-D-glucopyranose, and its methyl glycosides were surprisingly resistant to hydrolysis by either acid or emulsin.[276] Previous reports on the synthesis of 2-deoxy-2-fluoro-D-glucose (Vol. 3, p. 63) and the corresponding 2,4-difluoro-sugar (Vol. 4, p. 53) from appropriate anhydro-sugars have been amplified,[277] and 2-acetamido-2,6-dideoxy-6-fluoro-α-D-glucopyranose has been prepared by a standard nucleophilic displacement, using caesium fluoride in ethylene glycol, on the 6-sulphonate.[278]

Several fluorinated ketoses have been described for the first time. 1,6-Dideoxy-1,6-difluoro-D-fructose has been prepared from the 2,3-*O*-isopropylidene-1,6-di-*O*-toluene-*p*-sulphonate,[279] and the 1,2:4,5-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl derivative has been converted into 4-deoxy-4-fluoro-D-sorbose by way of an epoxide route.[280] Fluoride ion-displacement on 2,3:4,5-di-*O*-isopropylidene-1-*O*-methanesulphonyl-β-D-fructopyranose has allowed halogenation at position 1, but a similar

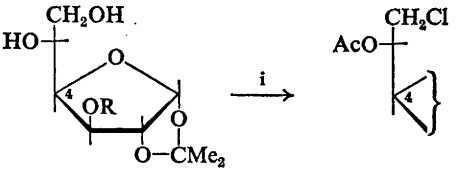
[271a] L. G. Revel'skaya, A. N. Anikeeva, and S. N. Danilov, *J. Gen. Chem.* (*U.S.S.R.*), 1972, **42**, 2300.
[272] J. Tomasic and D. Keglevic, *Croat. Chem. Acta*, 1972, **44**, 493 (*Chem. Abs.*, 1973, **79**, 115 820*r*).
[273] Y. Takagi, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 1261.
[274] G. Okada and E. J. Hehre, *Carbohydrate Res.*, 1973, **26**, 240.
[275] J. Podesva and J. Paćak, *Chem. listy*, 1973, **67**, 785 (*Chem. Abs.*, 1973, **79**, 115 787*k*).
[276] M. Černý, V. Prikrylova, and J. Paćak, *Coll. Czech. Chem. Comm.*, 1972, **37**, 2978.
[277] J. Paćak, J. Podesva, Z. Tocik, and M. Černý, *Coll. Czech. Chem. Comm.*, 1972, **37**, 2589.
[278] M. L. Shulman and A. Ya. Khorlin, *Carbohydrate Res.*, 1973, **27**, 141.
[279] J. Paćak, J. Halaskova, V. Štěphán, and M. Černý, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3646.
[280] M. Sarel-Imber and E. D. Bergmann, *Carbohydrate Res.*, 1973, **27**, 73.

displacement on 1,2:4,5-di-*O*-isopropylidene-3-*O*-methanesulphonyl-β-D-*ribo*-hexulopyranose was unsuccessful.[281]

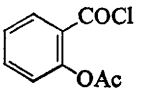
4,4′-Di- and 4,4′,6,6′-tetra-fluorinated derivatives of αα-trehalose have been described,[282] as has a 1,6-dideoxy-1,6-difluoro-D-mannitol derivative, which was subsequently converted into 1-deoxy-1-fluoro-L-glycerol and its 3-phosphate.[283]

Reference is made in Chapter 24 to the field desorption mass spectrometry of fluorinated D-glucose 6-phosphates.

α-Diols reacted with acetylsalicyloyl chloride in *p*-dioxan to give primary chlorides in a potentially useful method for the preparation of such compounds (Scheme 36),[284] but attempts to obtain analogues in the hexo-



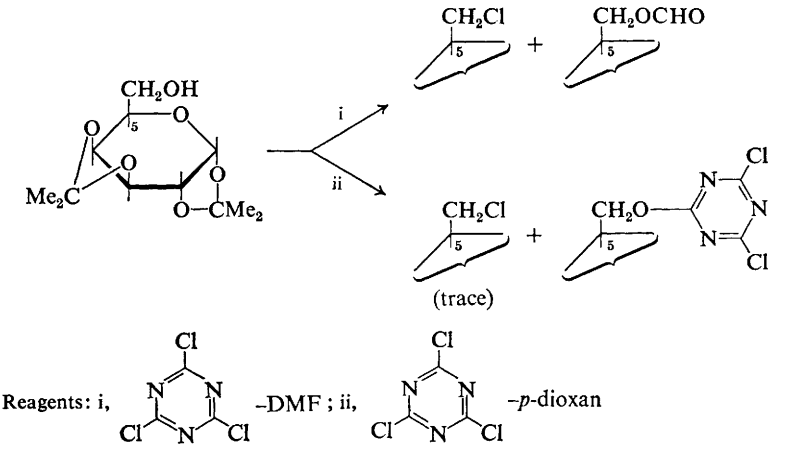
R = Ac, Ts, —CONHPh

Reagent: i,  —*p*-dioxan

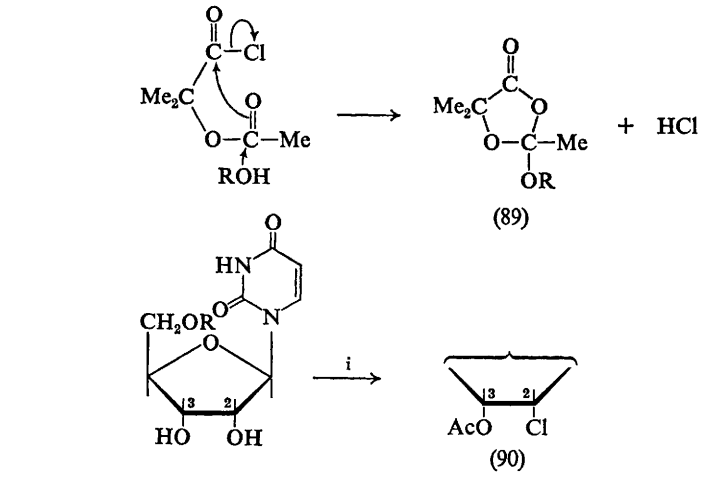
Scheme 36

pyranose series using cyanuric chloride were only partly successful. In DMF, a competing reaction gave formate esters, whereas triazinyl ethers were mainly produced in *p*-dioxan (Scheme 37).[285] Interesting developments in the nucleoside field have been reported from Moffatt's laboratory. Treatment of alcohols with 2-acetoxyisobutyryl chloride has been shown to give cyclic orthoester derivatives (89), whereas *cis*-diols on five- and six-membered rings furnish 2-acetoxy-1-chloro-derivatives. *trans*-Products were usually formed, but *cis*-products (90) were produced with 5′-substituted uridine derivatives (Scheme 38) following attack of the uracil carbonyl group on a 2′,3′-acetoxonium ion intermediate.[286] Similarities to the work described above with acetylsalicyloyl chloride are apparent. Reaction of 2-acetoxyisobutyryl chloride with adenosine yielded the chlorinated products indicated in Scheme 39; cordycepin was prepared

[281] J. G. Barnett and G. R. S. Atkins, *Carbohydrate Res.*, 1972, **25**, 511.
[282] L. Hough, A. K. Palmer, and A. C. Richardson, *J.C.S. Perkin I*, 1973, 784.
[283] W. J. Lloyd and R. Harrison, *Carbohydrate Res.*, 1973, **26**, 91.
[284] A. A. Akhrem, G. V. Zaitseva, and J. A. Mikhailopulo, *Carbohydrate Res.*, 1973, **30**, 223.
[285] A. Zamojski, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1973, **26**, 208.
[286] S. Greenberg and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1973, **95**, 4016.



Scheme 37

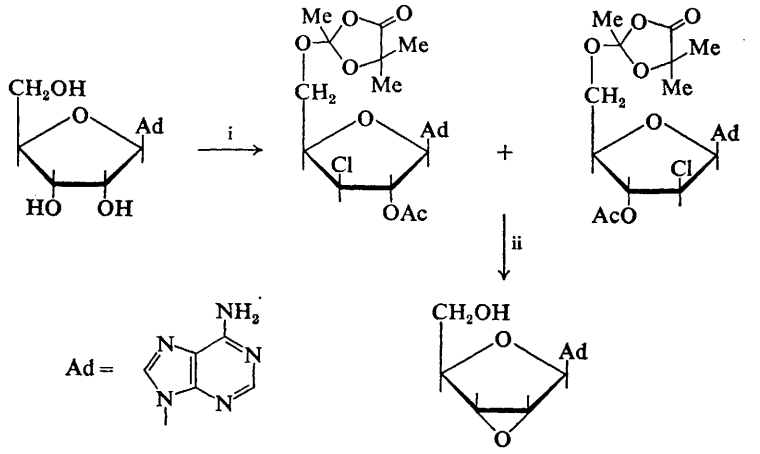


Reagent: i, $Me_2C(OAc)COCl$

Scheme 38

from the 3′-chloro-compound, whereas the 2′,3′-anhydride was obtainable from both products.[287] The reactions of tubercidin and formycin with 2-acetoxyisobutyryl chloride have also been described.[288] In closely related work, orthoesters have been used to obtain halogenated nucleosides,

[287] A. F. Russell, S. Greenberg, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1973, **95**, 4025.
[288] T. C. Jain, A. F. Russell, and J. G. Moffatt, *J. Org. Chem.*, 1973, **38**, 3179.

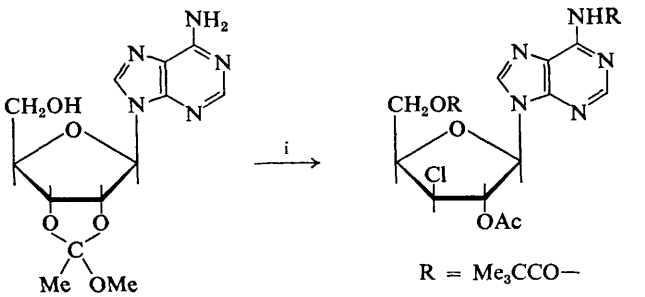


Reagents: i, $Me_2C(OAc)COCl$; ii, MeO^-

Scheme 39

which were then transformed by standard procedures into deoxy, epoxy, and unsaturated derivatives (Scheme 40).[179]

Derivatives of tervalent phosphorus have been used in displacement reactions to give halogeno- and thiocyanato-sugars.[289]



Reagent: i, $Me_3C{\cdot}COCl$–py

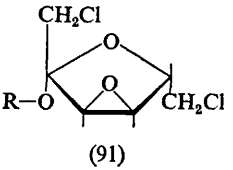
Scheme 40

Several chlorodeoxy-derivatives of disaccharides have been described. Treatment of methyl β-maltoside with sulphuryl chloride, followed by dechlorosulphation, furnished a 3,6,4′,6′-tetrachloro-disaccharide in which the chloro-groups at the secondary positions were introduced with inversion of configuration.[290] Methanesulphonyl chloride in DMF gave the same tetrachloro-compound directly, together with methyl 4-*O*-(6-chloro-6-

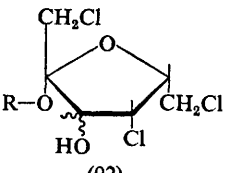
[289] E. E. Nifantév, M. P. Koroteev, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2095.

[290] P. L. Durette, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1973, **31**, 114.

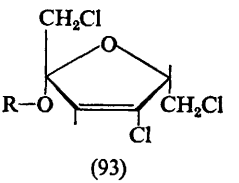
deoxy-α-D-glucopyranosyl)-3,6-dichloro-3,6-dideoxy-β-D-allopyranoside.[291] Hough's group have reported that treatment of sucrose with sulphuryl chloride at −78 °C gave 6-mono- and 6,6′-dichloro-derivatives, whereas several compounds, including (91), (92), and (93), were formed at elevated temperatures.[265, 292] 6-Mono- and 6,6′-di-halogeno-αα-trehaloses have been obtained by methods previously described.[293]



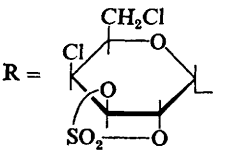
(91)



(92)

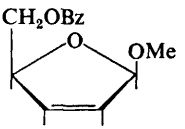


(93)

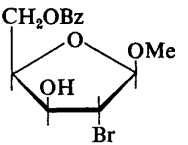


Secondary bromides can generally be prepared by displacement of sulphonyloxy-groups using lithium bromide in DMF.[294]

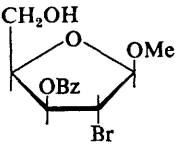
Many new bromodeoxy-sugars have been described. Addition of bromine in the presence of methanol and silver acetate to the pent-2-enofuranoside (94) gave the 2-bromoglycosides (95) and (96) by way of the



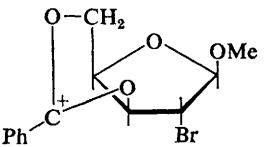
(94)



(95)



(96)

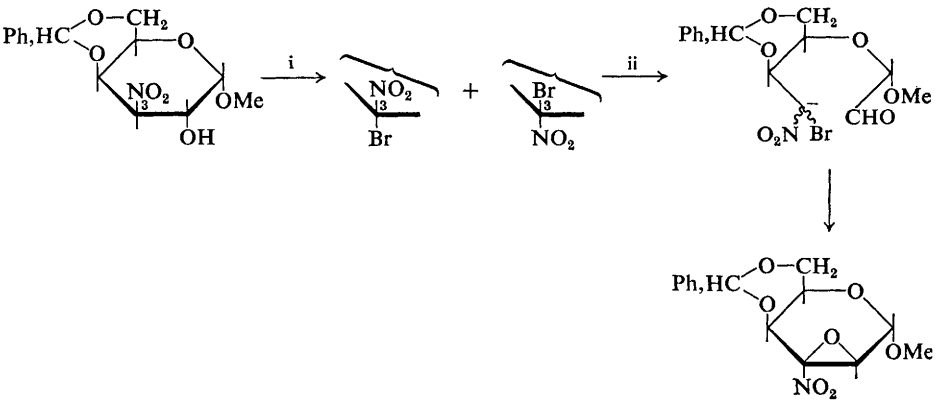


(97)

[291] R. G. Edwards, L. Hough, A. C. Richardson, and E. Tarelli, *Tetrahedron Letters*, 1973, 2369.
[292] J. M. Ballard, L. Hough, A. C. Richardson, and P. H. Fairclough, *J.C.S. Perkin I*, 1973, 1524.
[293] S. Hanessian and P. Lavallee, *Carbohydrate Res.*, 1973, **28**, 303.
[294] S. Inokawa, K. Yoshida, H. Yoshida, and T. Ogata, *Carbohydrate Res.*, 1973, **26**, 230.

benzoxonium ion (97).[295] 3′,5′-Di-*O*-benzoyl-2′-bromo-2′-deoxyuridine has been shown to undergo reductive debromination, with retention of configuration, when treated with tributyltin hydride; the stereochemistry of the reduction was established using a 2′-tritium-labelled precursor.[296]

An extensive report has appeared on geminally substituted halogenonitrosugars and on their subsequent conversion into epoxides. An outline of the results is depicted in Scheme 41, and it was proposed that, in the last step,

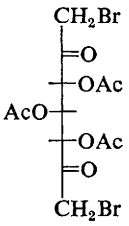


Reagents: i, NaOBr; ii, NaOH

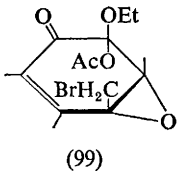
Scheme 41

epimerization at C-2 occurs after scission of the C-2–C-3 bond.[297] The 6- and 6′-mono- and 6,6′-di-bromosucroses have been prepared.[298]

Treatment of the 1,6-dibromo-compound (98) with sodium acetate in ethanol has been shown to give the cyclohexenone derivative (99)[299] (see also Chapter 19).



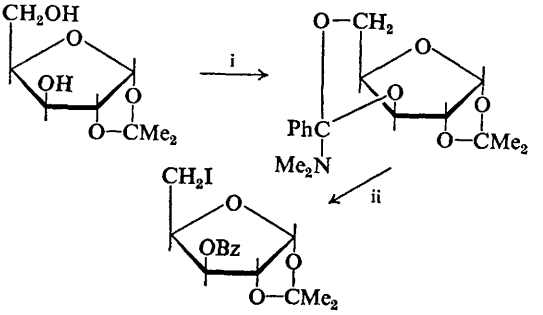
(98)



(99)

[295] R. G. S. Ritchie and W. A. Szarek, *Chem. and Ind.*, 1973, 530.
[296] S. David and C. Augé, *Carbohydrate Res.*, 1973, **28**, 125.
[297] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1973, **51**, 2001.
[298] L. Hough and K. S. Mufti, *Carbohydrate Res.*, 1973, **27**, 47.
[299] C. E. Cantrell and D. E. Kiely, *Tetrahedron Letters*, 1973, 4379.

A new method, analogous to the use of *N*-bromosuccinimide with benzylidene acetals, has been reported for the conversion of diols into bromo- and iodo-hydrin esters (Scheme 42).[300]



Reagents: i, $PhC(OEt)_2 \cdot NMe_2$; ii, *EtI–heat*

Scheme 42

Treatment of benzyl 2,3,4-tri-*O*-benzyl-6-*O*-toluene-*p*-sulphonyl-β-D-glucopyranoside with sodium iodide in DMF afforded the 6-iodo-compound, whereas the D-*galacto*-analogue yielded the 3,6-anhydride following 3-benzyloxy-group participation. This finding is consistent with the known difficulty of effecting the displacement of 6-sulphonyloxy-groups on D-galactopyranosides with external nucleophiles.[301]

[300] T. P. Culbertson, *J. Org. Chem.*, 1973, **38**, 3624.
[301] L. V. Volkova, M. G. Luchinskaya, N. G. Morozova, N. B. Rozanova, and R. P. Evstigneeva, *J. Gen. Chem.* (*U.S.S.R.*), 1972, **42**, 2101.

8
Amino-Sugars*

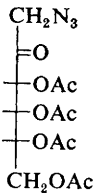
The synthesis of mono- and di-amino-glycosides of 2-deoxystreptamine has been reviewed (in German).[49]

Natural Products

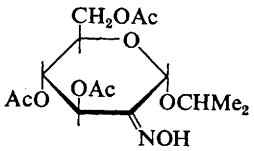
2-Acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose has been found to be released from hog submaxillary glycoprotein by an oligosaccharidase from *Clostridium perfringens*,[302] and 4-acetamido-4,6-dideoxy-D-glucose and -D-galactose have been identified as components of nucleotides obtained from *Escherichia coli*.[303]

Synthesis

A number of 1-amino-1-deoxy-D-psicose derivatives have been prepared by way of the azido-derivative (100), which was derived from the corresponding 1-bromo-1-deoxy-compound; methanolysis of (100) gave the methyl furanosides, which were converted into 1-acetamido-1-deoxy-compounds.[304]



(100)



(101)

Reduction of either the oximo-α-glycoside (101) or the corresponding *O*-acetyloxime has been studied using such reagents as palladium–hydrogen in the presence of hydrazine, diborane in THF, and lithium aluminium hydride in THF.[305] The first two reagents gave predominantly the D-*gluco*-

[302] C. C. Huang and D. Aminoff, *J. Biol. Chem.*, 1972, **247**, 6737.
[303] D. N. Dietzler and J. L. Strominger, *J. Biol. Chem.*, 1973, **248**, 104.
[304] H. Hřebabecký, J. Krupička, and J. Farkaš, *Coll. Czech. Chem. Comm.*, 1973, **38**, 3181.
[305] R. U. Lemieux, K. James, T. L. Nagabhushan, and Y. Ito, *Canad. J. Chem.*, 1973, **51**, 33.

* See also Part I, Chapter 20.

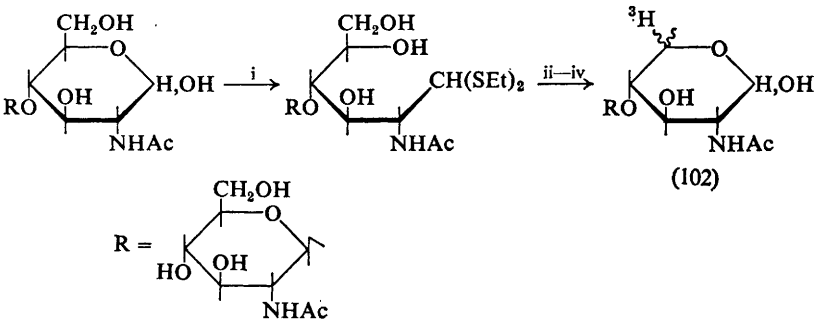
amine, whereas the latter reagent afforded mainly the D-*manno*-amine. This procedure provides a highly stereoselective synthesis of 2-amino-2-deoxy-α-D-glucopyranosides, although similar reductions of the *lyxo*-analogue of (101) were not as stereoselective. The method was also used in the synthesis of derivatives of 2-amino-2-deoxy-disaccharides.[91, 305]

Perry's group has reported syntheses of 2-amino-2,6-dideoxyhexoses possessing the D (and L)-*galacto*-, D (and L)-*talo*-, D-*allo*-, D-*altro*-, L-*gluco*-, and L-*manno*-configurations.[306-308]

Derivatives of 2-acetamido-2,3-dideoxyhexoses have been prepared from related 2,3-unsaturated sugars (see also Chapter 14).[309] 4,6-*O*-Isopropylidene derivatives of a number of 2-acetamido-2-deoxy-D-hexoses have been reported [201, 202] (see Chapter 5), and the formation of 3,6-anhydro-derivatives of 2-acylamino-2-deoxyhexosides is referred to in Chapter 4 (see Schemes 23 and 24).[193] Koenigs–Knorr syntheses of glycosides of amino-sugar derivatives have been studied in some detail [68] (see Chapter 3).

αα-Trehalose has been transformed into disaccharides in which either one or both of the sugar rings have a 2-benzylamino-2-deoxy-D-*altro*-structure.[310] 6-Acetamido-6-deoxylactose has been prepared from 1,6-anhydrolactose (lactosan).[186]

The tritium-labelled disaccharide (102) has been obtained by the sequence of reactions shown in Scheme 43, and it was also isolated from a transfer reaction, catalysed by lysozyme, involving tetra-*N*-acetylchitotetraose and 2-acetamido-2-deoxy-D-[5-^{3}H]xylose.[311] 2-Amino-2-deoxy-D-[2-^{14}C]glucose and 2-[^{15}N]amino-2-deoxy-D-glucose have been synthesized by standard methods.[312]

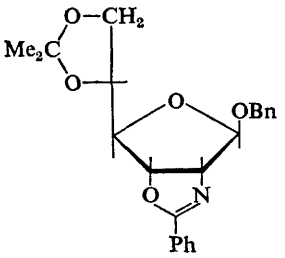


Reagents: i, EtSH–HCl; ii, IO_4^-; iii, NaB^3H_4; iv, $HgCl_2$–H_2O

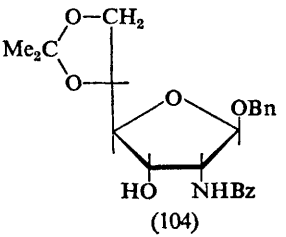
Scheme 43

[306] M. B. Perry and V. Daoust, *Canad. J. Chem.*, 1973, **51**, 974.
[307] M. B. Perry and V. Daoust, *Carbohydrate Res.*, 1973, **31**, 131.
[308] M. B. Perry and V. Daoust, *Carbohydrate Res.*, 1973, **27**, 460.
[309] N. Pravdic, B. Zidovec, I. Franjic, and H. G. Fletcher, jun., *Croat. Chem. Acta*, 1973, **45**, 343 (*Chem. Abs.*, 1973, **79**, 79 095x).
[310] I. Jezo, *Chem. Zvesti*, 1973, **27**, 381 (*Chem. Abs.*, 1973, **79**, 92 514c).
[311] P. van Eikeren, W. A. White, and D. M. Chipman, *J. Org. Chem.*, 1973, **38**, 1831.
[312] U. Hornemann, *Carbohydrate Res.*, 1973, **28**, 171.

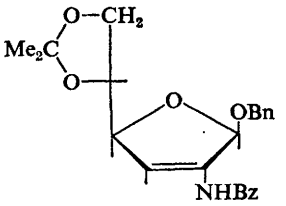
Opening of the oxazoline ring in (103) with toluene-*p*-sulphonic acid in aqueous pyridine furnished the expected *cis*-benzamido-alcohol (104), whereas both (104) and the enamide (105) resulted when the oxazoline was



(103)



(104)



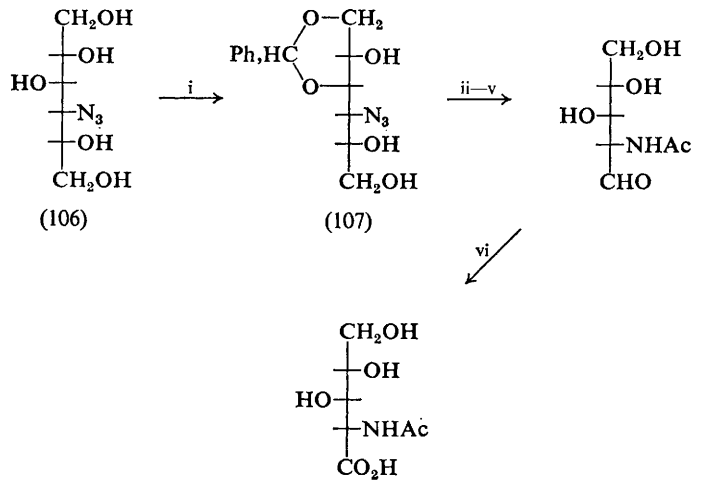
(105)

treated with potassium t-butoxide in DMSO.[313] A synthesis of polyoxamic acid (2-amino-2-deoxy-L-xylonic acid) has been achieved; methyl 4-azido-4-deoxy-α-D-glucopyranoside was first converted into 3-azido-3-deoxy-L-gulitol (106), whereafter the synthesis proceeded as shown in Scheme 44.[314] As well as the expected product of glycol cleavage, oxidation of (107) with periodate ion yielded an unsaturated compound, presumably resulting from labilization of the proton adjacent to the aldehydic group in the initial product of oxidation.

A number of simple derivatives of gentosamine (3-amino-3-deoxy-D-xylose) have been prepared by ring opening of methyl 2,3-anhydro-β-D-ribopyranoside with the azide ion.[315] Ring opening of methyl 2,3-anhydro-4-*O*-benzoyl-6-deoxy-α-D-allopyranoside (108) with ammonia yielded the 3-amino-3-deoxy-D-glucoside (109), as the major product, and the 2-amino-2-deoxy-D-altroside (110).[316]

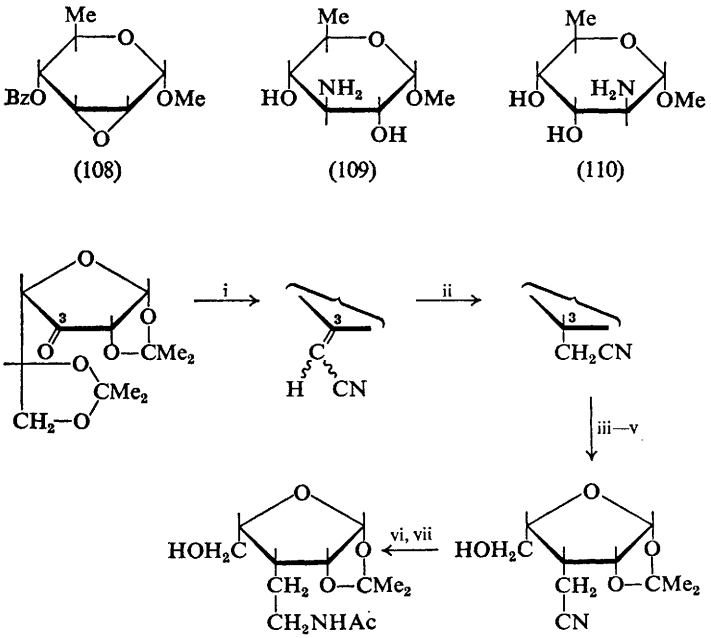
3-*C*-(2-Acetamidoethyl)-3-deoxy-1,2-*O*-isopropylidene-β-L-lyxofuranose has been obtained by way of a Wittig reaction on 1,2:5,6-di-*O*-isopro-

[313] P. A. Gent, R. Gigg, S. May, and R. Conant, *J.C.S. Perkin I*, 1972, 2748.
[314] H. Kuzuhara, H. Ohrui, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 949.
[315] A. Hasegawa, C. Yoshida, and S. Kosuge, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 1971, **31**, 209 (*Chem. Abs.*, 1973, **78**, 111 656y).
[316] H. H. Baer and S.-H. Lee Chiu, *Carbohydrate Res.*, 1973, **28**, 390.



Reagents: i, PhCHO–HCl; ii, H_2–Ni; iii, Ac_2O–MeOH; iv, IO_4^-; v, H^+; vi, Br_2–H_2O

Scheme 44

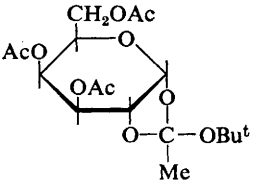


Reagents: i, $(EtO)_2 \cdot PO \cdot CH_2CN$; ii, H_2–Pd; iii, H^+; iv, IO_4^-; v, $NaBH_4$; vi, H_2–Pt; vii, Ac_2O–EtOH

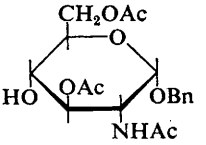
Scheme 45

pylidene-α-D-*xylo*-hexofuranos-3-ulose as shown in Scheme 45,[317] and derivatives of 3-amino-3,4,6-trideoxy-D- and -L-*xylo*-hexopyranose have been described.[318]

N-Acetyl-lactosamine has been synthesized from lactal hexa-acetate by way of addition of nitrosyl chloride to the alkenic linkage.[319] It was also prepared by condensation of the orthoester (111) with the alcohol (112), followed by de-protection of the product disaccharide.[319a]



(111)



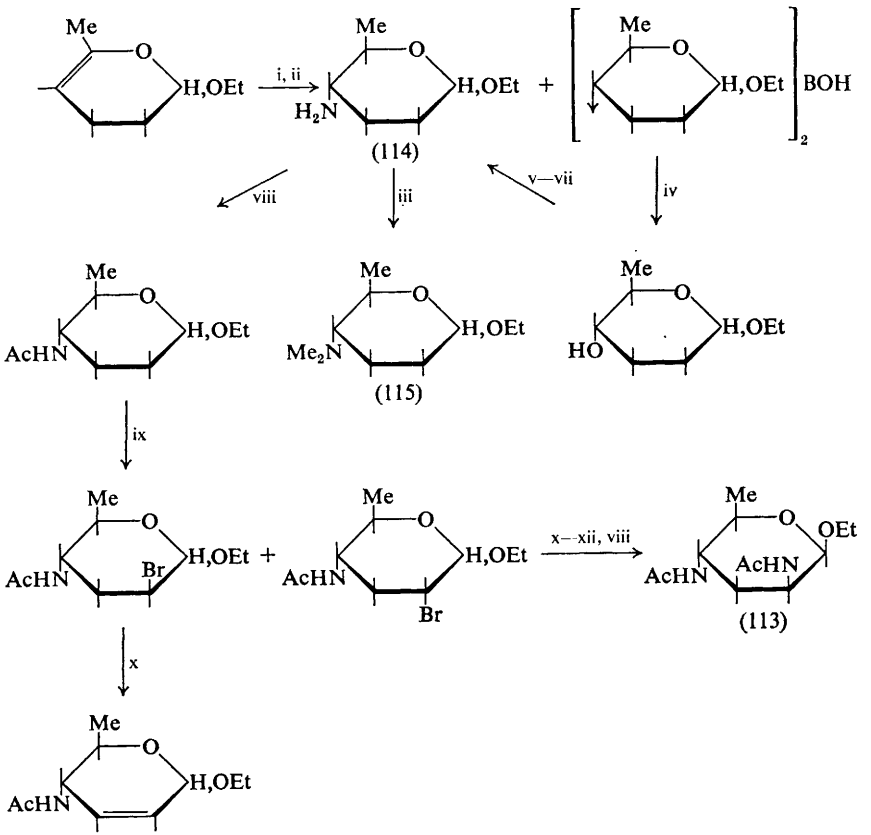
(112)

Syntheses of ethyl *NN'*-diacetyl-β-D-kasugaminide (113), ethyl αβ-DL-tolyposaminide (114), and ethyl αβ-DL-forosaminide (115) have been accomplished from a common precursor by the reactions illustrated in Scheme 46.[320] An *N*-benzoyl derivative of tolyposamine (4-amino-2,3,4,6-tetra-deoxy-L-*erythro*-hexose) has also been synthesized by the reactions shown in Scheme 47.[321, 322]

6'-Amino-6'-deoxymaltose has been prepared following enzymic hydrolysis of 6-azido-6-deoxycylohexa-amylose and catalytic reduction of the released disaccharide.[246] Sucrose derivatives bearing diethylamino- and piperidino-groups have resulted from appropriate nucleophilic displacements on 1,6,6'-tri-*O*-toluene-*p*-sulphonylsucrose, and 6-deoxy-6-piperidino-6'-*O*-toluene-*p*-sulphonylsucrose was similarly obtained from the corresponding disulphonate.[323]

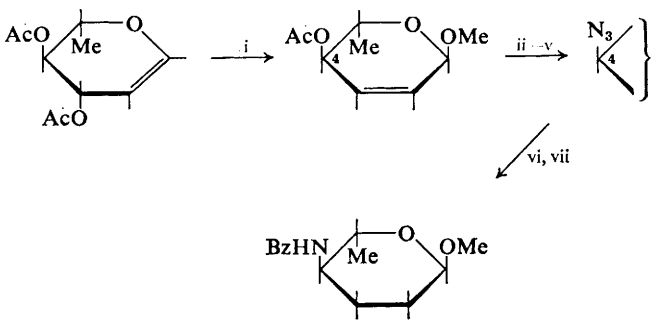
N-Acetyl-4-deoxy- and *N*-acetyl-4,6-dideoxy-muramic acids have been prepared by reaction of *N*-acetylmuramic acid with sulphuryl chloride, followed by reduction of the resulting chloro-derivatives with tri-n-butyltin hydride.[324]

[317] A. Rosenthal and D. A. Baker, *Carbohydrate Res.*, 1973, **26**, 163.
[318] K. Kefurt, K. Čapek, J. Čapková, Z. Kefurtová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1972, **37**, 2985.
[319] B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2365.
[319a] S. E. Zurabjan, E. N. Lopantseva, and A. Ya. Khorlin, *Doklady Akad. Nauk S.S.S.R.*, 1973, **210**, 1216.
[320] S. Yasuda, T. Ogasawara, S. Kawabata, I. Wataki, and T. Matsumoto, *Tetrahedron*, 1973, **29**, 3141.
[321] J. S. Brimacombe, L. W. Doner, A. J. Rollins, and A. K. Al-Radhi, *Tetrahedron Letters*, 1973, 87.
[322] J. S. Brimacombe, L. W. Doner, A. J. Rollins, and A. K. Al-Radhi, *J.C.S. Perkin I*, 1973, 1295.
[323] R. Neumann and J. A. Ibarra, *J. prakt. Chem.*, 1972, **314**, 365.
[324] H. Arita, K. Fukukawa, and Y. Matsushima, *Bull. Chem. Soc. Japan*, 1972, **45**, 3611.



Reagents: i, B_2H_6; ii, NH_2Cl; iii, HCO_2H–HCHO; iv, H_2O_2–NaOH; v, CrO_3; vi, NH_2OH; vii, Na–EtOH; viii, Ac_2O; ix, Br_2–HCl–EtOH; x, NaN_3; xi, H_2–Pt; xii, resolution *via* tartrate

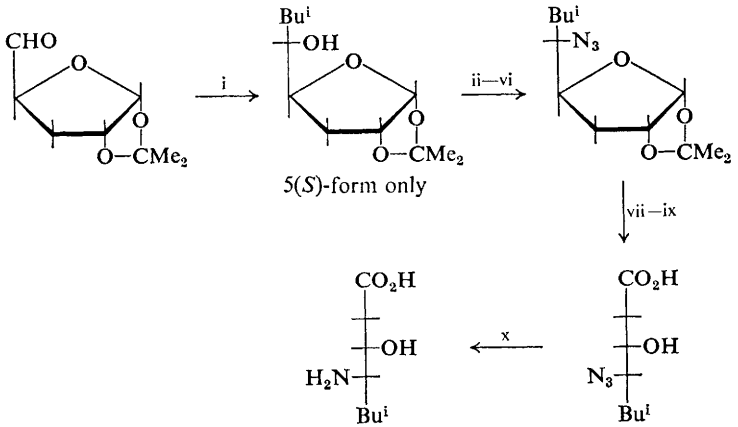
Scheme 46



Reagents: i, BF_3–MeOH; ii, MeONa–MeOH; iii, MsCl–py; iv, NaI; v, NaN_3; vi, H_2–Pt; vii, Bz_2O

Scheme 47

The absolute configuration of the 4-amino-3-hydroxy-6-methylheptanoic acid derived from pepstatin A has been established by its synthesis from a monosaccharide precursor (Scheme 48).[325]



Reagents: i, $Me_2CH{\cdot}CH_2MgBr$; ii, MsCl–py; iii, BzO^-; iv, MeO^-; v, TsCl–py; vi, NaN_3–DMF; vii, aq. AcOH; viii, $NaIO_4$; ix, NaOI; x, H_2–Pd

Scheme 48

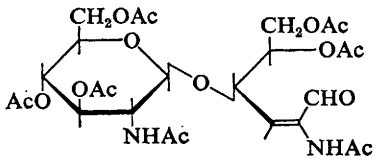
Reactions

The partial acetylation of methyl 4-acetamido-6-*O*-acetyl-4-deoxy-α-D-glucopyranoside (60) has been examined [210] (see Chapter 6). A variety of acylamino-derivatives (RCONH) of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside have been prepared for use as substrates in enzymic studies; these have included derivatives where R = H, Et, Pr^i, Pr^n, and Ph,[326] and also where R is a mono-, di, or tri-halogenoacetyl group containing fluoro-, chloro-, or bromo-substituents.[327] It was necessary to use the more acid-labile 4,6-*O*-(*p*-methoxybenzylidene) blocking group in a synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside, since the aglycone is cleaved under the acid conditions required to remove an unsubstituted 4,6-*O*-benzylidene group.[328]

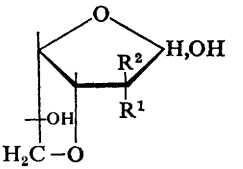
A minor product formed in the acetolysis of chitin to chitobiose octaacetate has been identified as the unsaturated disaccharide (116). The disaccharide was also obtained under conditions of acetolysis from di-*N*-acetylchitobiose methyl glycoside and tri-*N*-acetylchitotriose, but not from di-*N*-acetylchitobiose itself, suggesting that it is probably an artefact of the work-up procedure.[329]

[325] M. Kinoshita, S. Aburaki, A. Hagiwara, and J. Imai, *J. Antibiotics*, 1973, **26**, 249.
[326] K. Yamamoto, *J. Biochem. (Japan)*, 1973, **73**, 631.
[327] K. Yamamoto, *J. Biochem. (Japan)*, 1973, **73**, 749.
[328] K. Yamamoto, *Bull. Chem. Soc. Japan*, 1973, **46**, 658.
[329] E. W. Thomas, *Carbohydrate Res.*, 1973, **26**, 225.

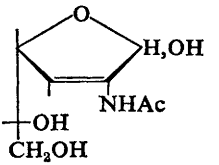
Alkaline degradation of 2-acetamido-2-deoxy-D-galactose yielded, *inter alia*, 2-acetamido-3,6-anhydro-2-deoxy-D-gulose (117) and -D-idose (118), and it was suggested that these anhydro-sugars are formed by an intramolecular attack of HO-6 on the alkenic bond of the chromogen (119).[330]



(116)



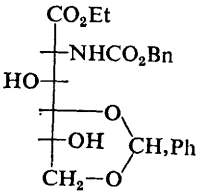
(117) R^1 = NHAc, R^2 = H
(118) R^1 = H, R^2 = NHAc



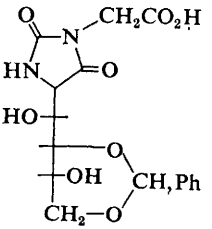
(119)

Hydrolysis of the *N*-carbobenzyloxy-derivative (120) with alkali has been shown to give the hydantoin derivative (121).[331]

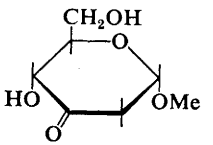
Optimum conditions have been established for the isolation of 2,5-anhydro-D-mannitol by deamination of 2-amino-2-deoxy-D-glucose and D-glucosaminides, followed by reduction with buffered borohydride.[332] When *O*-acetylated derivatives of 2-amino-2-deoxy-D-glucose were used, the products of deamination were found to include 5-(acetoxymethyl)-2-furaldehyde and acetates of D-glucose and D-mannose, as well as 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannitol. Deamination of methyl 2-amino-2-deoxy-α-D-mannopyranoside with nitrous acid afforded (122) and (123),



(120)



(121)

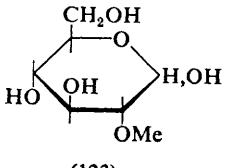


(122)

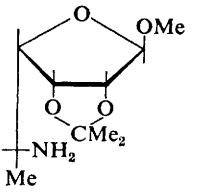
[330] V. A. Derevitskaya, L. M. Likhosherstov, V. A. Schennikov, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **26**, 201.
[331] G. Deak, E. Zara-Kaczian, and L. Kisfaludy, *Acta Chim. Acad. Sci. Hung.*, 1973, **75**, 185.
[332] D. Horton and K. D. Philips, *Carbohydrate Res.*, 1973, **30**, 367.

in the ratio of 2 : 1, as the products of hydride and methoxy-group migration, respectively; the mechanism of this reaction was discussed in some detail and comparison was made with the corresponding deamination of 2-amino-2-deoxy-D-mannose.[333]

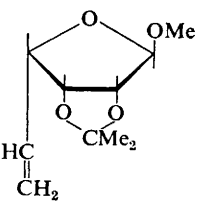
The products resulting from deamination of 2-amino-2-deoxy-D-glucitol with nitrous acid, followed by borohydride reduction, have been identified



(123)



(124)



(125)

as 2-deoxy-D-*arabino*- and -D-*ribo*-hexitols, 2-deoxy-2-hydroxymethyl-D-*arabino* (or *ribo*)-pentitol, and D-mannitol.[334] The products obtained directly from the deamination, following chromatography on an ion-exchange resin, were 2-deoxy-D-*arabino*-hexose, 2-deoxy-D-*erythro*-hex-3-ulose, 2-deoxy-2-hydroxymethyl-D-*arabino* (or *ribo*)-pentose, and D-mannitol; these products can be accounted for by rearrangement or solvolysis of the initially formed carbonium ion. Deamination of the 5-amino-5-deoxyglycoside (124) with nitrous acid in aqueous acetic acid has been found to yield the terminal unsaturated sugar (125) among the products.[252]

The syntheses of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*N*-nitroso)acetamido-α- and -β-D-glucopyranoses have been described.[335] Decomposition of the α-nitroso-amide in chloroform containing a trace of ethanol at room temperature afforded β-D-glucopyranose penta-acetate and ethyl β-D-glucopyranoside tetra-acetate as major products, arising from attack on an intermediate acetoxonium ion. Decompositions of both α- and β-nitroso-amides in aqueous acetone, however, gave mainly 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose, which is considered to be formed from a bicyclic oxonium ion arising from participation of the ring-oxygen atom in heterolysis of the diazonium ion.

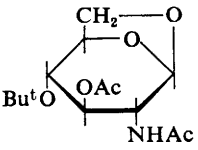
It has been shown that the *N*-benzamido-group can be converted into the *N*-acetamido-group on refluxing with acetic anhydride and acetic acid.[164] A procedure has been developed for the quantitative de-*N*-acetylation of amino-sugars using hydrazine in the presence of hydrazine sulphate.[109, 110]

A number of 2-deoxy-2-methylaminoglycosides have been prepared from the appropriate diethyl dithioacetal; the *N*-methyl-group was introduced by Kuhn methylation of a sugar oxazolidinone, which was subsequently

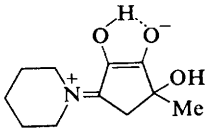
[333] J. W. Llewellyn and J. M. Williams, *J.C.S. Perkin I*, 1973, 1997.
[334] T. Bando and Y. Matsushima, *Bull. Chem. Soc. Japan*, 1973, **46**, 593.
[335] J. W. Llewellyn and J. M. Williams, *Carbohydrate Res.*, 1973, **28**, 339.

opened with base.[336] It was noted that certain amides containing comparatively bulky substituents in the vicinity of the NH-group were resistant to methylation, and factors affecting *N*-methylation with the Kuhn reagent were discussed.

Derivatives of 2-acetamido-1,6-anhydro-2-deoxy-D-glucose have been used as intermediates in the synthesis of disaccharides having 2-amino-2-deoxy-D-glucose at the reducing end.[337] The key intermediate (126) could be converted into compounds possessing a free hydroxy-group at C-3 or C-4 by treatment with either sodium methoxide or trifluoroacetic acid, respectively.



(126)



(127)

6-Deoxy-6-piperidino-D-glucose was transformed into (127) on treatment with piperidine or tertiary amines in aqueous acetic acid.[338]

Physical Measurements

The mass spectra of *N*-salicylidene derivatives of amino-sugars have been discussed in some detail.[339] ^{13}C N.m.r. studies have been performed on 2-acetamido-2-deoxy-D-hexoses and a number of 3-*O*-acetyl and 1-phosphate derivatives thereof.[340]

Di- and Tri-amino-sugars

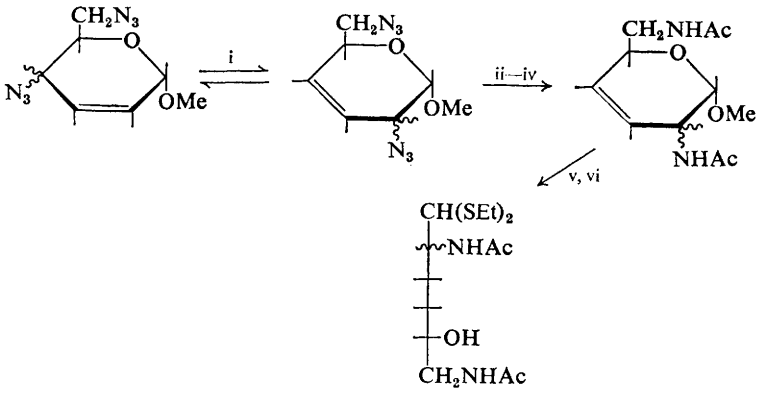
N-Acetylbacillosamine has been identified as 4-acetamido-2-amino-2,4,6-trideoxy-D-glucose[341] and a synthesis (by standard methods) of the 2,4-diacetamido-sugar has been described.[341a] The isomeric sugars 2,4-diacetamido-2,4,6-trideoxy-L-altrose, -L-idose, and -L-talose have also been synthesized (from benzyl 6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside) in connection with this study.[342]

Both the 3- and 4-methanesulphonyloxy-groups were displaced when (79) was solvolysed in wet 2-methoxyethanol, presumably with participation of the 2- and 6-acetamido-groups, respectively, to give methyl 2,6-diacetamido-2,6-dideoxy-α-D-gulopyranoside (80).[250]

[336] P. A. J. Gorin, *Carbohydrate Res.*, 1973, **27**, 309.
[337] Y. Rabinsohn, A. J. Acher, and D. Shapiro, *J. Org. Chem.*, 1973, **38**, 202.
[338] R. Neumann and G. Henseke, *Z. Chem.*, 1973, **13**, 99.
[339] S. Inouye, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2320.
[340] D. R. Bundle, H. J. Jennings, and I. C. P. Smith, *Canad. J. Chem.*, 1973, **51**, 3812.
[341] U. Zehavi and N. Sharon, *J. Biol. Chem.*, 1973, **248**, 433.
[341a] A. Liav, J. Hildesheim, U. Zehavi, and N. Sharon, *J.C.S. Chem. Comm.*, 1973, 668.
[342] A. Liav and N. Sharon, *Carbohydrate Res.*, 1973, **30**, 109.

The diethyl dithioacetals of 2,6-diacetamido-2,3,4,6-D-*erythro*-hexose (purpurosamine C) and the D-*threo*-isomer (*epi*-purpurosamine C) have been synthesized by the route shown in Scheme 49.[343] The sequence depicted in Scheme 50 was used to prepare a derivative of 2,3-diamino-2,3-dideoxy-D-ribose.[344]

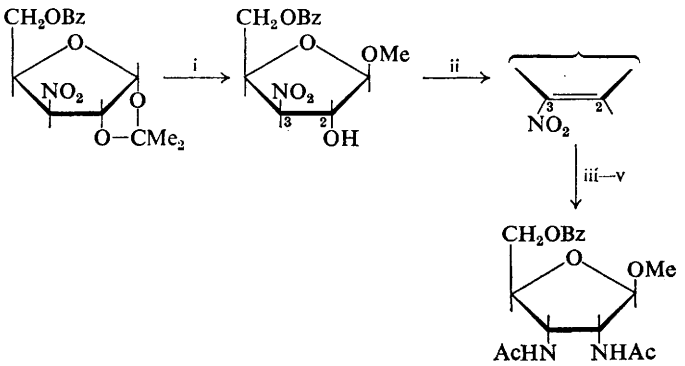
A number of modified αα-trehaloses bearing azido- and amino-groups have been reported,[345] and 1′,6,6′-triamino-1′,6,6′-trideoxysucrose has been



(purpurosamine C has the D-*erythro*-configuration and *epi*-purpurosamine C has the D-*threo*-configuration)

Reagents: i, heat; ii, N_2H_4–Ni; iii, Ac_2O; iv, separation by preparative t.l.c.; v, H_2–Pt; vi, EtSH–HCl

Scheme 49



Reagents: i, MeOH–H_2SO_4; ii, Ac_2O–py; iii, HN_3–$CHCl_3$; iv, H_2–Pd; v, Ac_2O–MeOH

Scheme 50

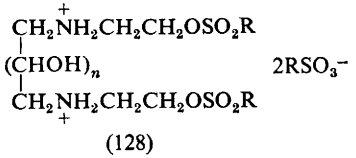
[343] J. Cleophax, J. LeBoul, A. Olesker, and S. D. Gero, *Tetrahedron Letters*, 1973, 4911.

[344] T. Takamoto, H. Tanaka, and R. Sudoh, *Chem. Letters*, 1972, 1125 (*Chem. Abs.*, 1973, **78**, 16 395*d*).

[345] L. Hough, P. A. Munroe, A. C. Richardson, Y. Ali, and S. T. K. Bukhari, *J.C.S. Perkin I*, 1973, 287.

prepared from the corresponding tri-*O*-toluene-*p*-sulphonate by way of an azide displacement.[346]

A series of compounds of the general formula (128) have been synthesized and tested for cytostatic activity; compounds with R = Me and $n = 2$ (*erythro* or *threo*) and with R = *p*-$NO_2C_6H_4$ and $n = 2$ (*threo*) exhibited substantial activity.[347]

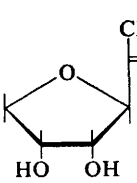


(128)

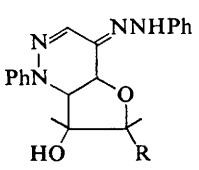
[346] R. Khan, K. S. Mufti, and M. R. Jenner, *Carbohydrate Res.*, 1973, **30**, 183.
[347] T. Horvath and L. Vargha, *Magyar Kém. Lapja*, 1972, **27**, 361.

9
Hydrazones and Osazones

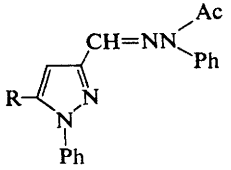
The mass spectra of Diels 3,6-monoanhydro-osazones [*e.g.* (129)], Percival dianhydro-osazones [*e.g.* (130)], and dianhydro-osazones of the pyrazole type [*e.g.* (131)] show distinctly characteristic fragmentation patterns.[348]



(129)

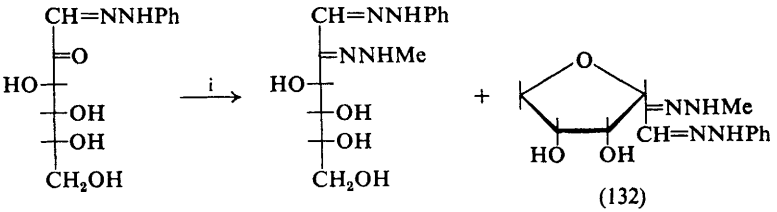


(130) R = CH_2OH or H



(131) R = H or ·$CHOHCH_2OH$

Mass spectroscopic and c.d. and n.m.r. evidence has been presented for the formation, *inter alia*, of 3,6-anhydro-D-*arabino*-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (132) when D-*arabino*-hexosulose 1-(phenylhydrazone) reacts with methylhydrazine (Scheme 51), although



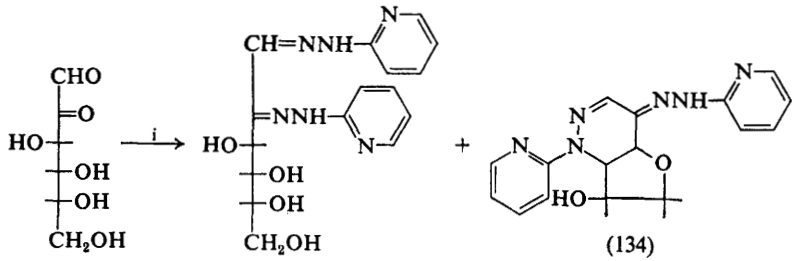
(132)

Reagent: i, $MeNHNH_2$

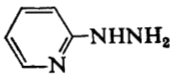
Scheme 51

the D-*ribo*-configuration seems more logical on mechanistic and steric grounds. Treatment of D-*arabino*-hexosulose with 2-hydrazinopyridine was shown to give the osazone (133) and a dianhydro-osazone (134) of the Percival type (Scheme 52).

[348] H. S. El Khadem, R. J. Sindric, and El Sayed H. El Ashry, *Carbohydrate Res.*, 1973, **30**, 165.

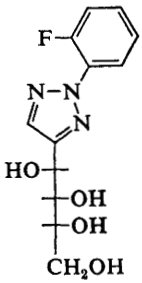


(133) (134)

Reagent: i, 

Scheme 52

The effects of various acids in catalysing the formation of D-*arabino*-hexosulose phenylosazone have been investigated, and a ketimine intermediate was proposed.[349] D-*arabino*-Hexulose (*o*-fluorophenyl)osotriazole (135) has been obtained by reaction of D-glucose with (*o*-fluorophenyl)-hydrazine, followed by treatment of the resulting osazone with copper(II) sulphate.[350] (N.B. Similar treatment of *o*-bromo-, *o*-chloro-, or *o*-iodo-phenylosazones resulted in loss of the halogen.)



(135)

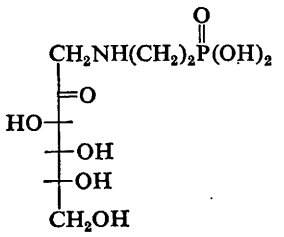
[349] V. A. Afanas'ev and I. F. Strel'tsova, *Zhur. fiz. Khim.*, 1972, **46**, 2545.
[350] H. S. El Khadem and D. L. Swartz, *Carbohydrate Res.*, 1973, **30**, 400.

10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines and Related Compounds

Glycosylamines have been shown to be inhibitors of the corresponding glycosidases.[351] Reduction of the pyridinium salts of fully acetylated *N*-glycosides of maltose and lactose with sodium borohydride gave acetylated *N*-aldosyl 1,4-dihydropyridines.[352] *N*-Glycosides of *p*-aminoacetophenone oxime have been prepared,[353] and the kinetics of the condensation of 3,4-xylidine with pentoses have been examined.[354]

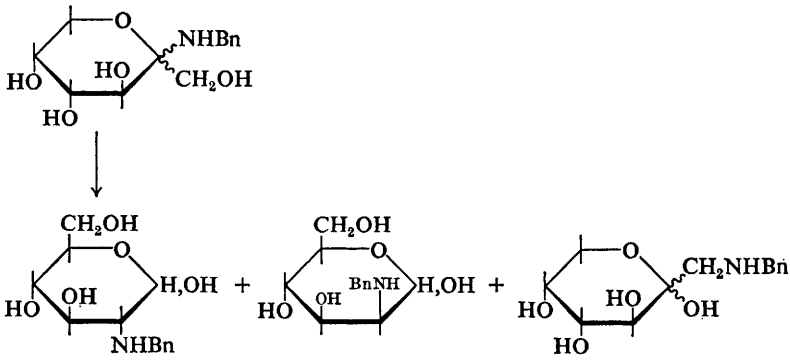
1-*N*-(L-Aspart-4-oyl)-β-glycopyranosylamines of 2-acetamido-2-deoxy-D-galactose, D-glucose, and D-mannose have been prepared by standard methods, and the stability of the compounds in acidic and alkaline media was examined.[355] Treatment of D-glucose with 2-aminoethylphosphonic acid initially gave the glycosylamine, which then underwent Amadori rearrangement to 1-deoxy-1-(2-phosphonoethylamino)-D-fructose (136).[356]



(136)

The mutarotation of *N*-aryl-D-fructopyranosylamines has been shown to lead to products that are probably the isomeric furanosylamines.[357] Moreover, ketosylamines can also be isomerized to the corresponding epimeric 2-amino-2-deoxyaldoses and aminoaldulose (Scheme 53) (the latter reaction is sometimes referred to as the 'Heyns rearrangement'). Further work on

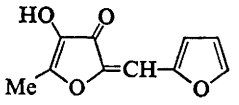
[351] H.-Y. L. Lai and B. Axelrod, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 463.
[352] A. Piskorska-Chlebowska, *Roczniki Chem.*, 1972, **46**, 2341.
[353] J. Sykulski, *Acta Polon. Pharm.*, 1972, **29**, 555.
[354] S. Kolka, *Zerz. Nauk Wydz. Met. Fiz. Chem. Univ. Gdenski. Chem.*, 1971, **1**, 149.
[355] M. Tanaka and I. Yamashina, *Carbohydrate Res.*, 1973, **27**, 175.
[356] S. K. Das, J. L. Abernethy, and L. D. Quin, *Carbohydrate Res.*, 1973, **30**, 379.
[357] K. Heyns and W. Beilfuss, *Chem. Ber.*, 1973, **106**, 2680.



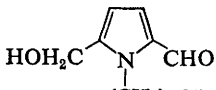
Scheme 53

the rearrangements of aldosyl- and ketosyl-amines of weak bases (*e.g.* *p*-nitroaniline) has shown that aldosylamines generally give the Amadori product, whereas ketosylamines give more complex mixtures, as typified in Scheme 53.[358]

Pentoses gave the Maillard product (137) on heating with isopropylamine and acetic acid.[359] The heterocyclic derivatives (138) and (139) were obtained



(137)



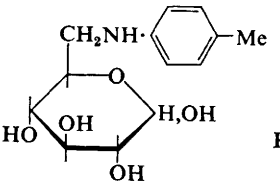
(138) R = H
(139) R = Me

from Maillard reactions of D-glucose and ethanolamine conducted in either methanol or aqueous methanol.[360]

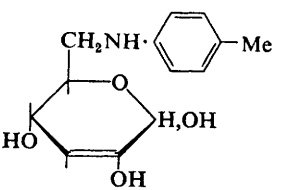
Hydrolysis of *N*-substituted 6-amino-6-deoxy-D-glucopyranosylamines with aqueous acetic acid afforded such derivatives as (140), whereas treatment with either secondary or tertiary amines yielded products of dehydration [*e.g.* (141)] and browning.[361]

Treatment of octa-*O*-benzoyl-lactose with methanolic ammonia gave, in addition to lactose, 1,1-bis(benzamido)-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol and *N*-benzoyl-4-*O*-β-D-galactopyranosyl-D-glucopyranosylamine.[362] The $^4C_1$ conformation of 1-acylamino-β-D-lyxopyranosylamines

[358] K. Heyns and W. Beilfuss, *Chem. Ber.*, 1973, **106**, 2693.
[359] Th. Severin and U. Kroenig, *Chem. Mikrobiol. Technol. Lebensm.*, 1972, **1**, 156 (*Chem. Abs.*, 1972, **77**, 152471g).
[360] H. Kato and M. Fujimaki, *Lebensm.-Wiss. Technol.*, 1972, **5**, 172.
[361] R. Neumann and G. Henseke, *Z. Chem.*, 1973, **13**, 58.
[362] J. O. Deferrari, I. M. E. Thiel, and R. A. Cadenas, *Carbohydrate Res.*, 1973, **29**, 141.



(140)



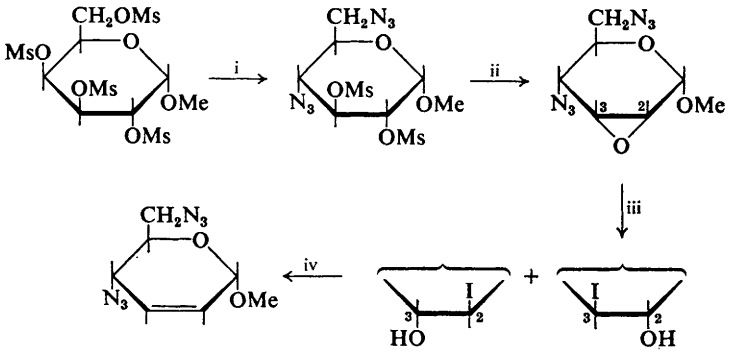
(141)

has been indicated to be more stable than the $^1C_4$ form by $\gtrsim 2$ kcal mol^{-1}, owing to the operation of the reverse anomeric effect.[363]

The preparations of partially acetylated maltosyl and lactosyl pyridinium bromides and perchlorates have been reported.[364]

Azido-sugars

Methyl 4,6-diazido-2,3,4,6-tetradeoxy-α-D-*erythro*-hex-2-enopyranoside has been derived from methyl 2,3,4,6-tetra-*O*-methanesulphonyl-α-D-galactopyranoside by the reactions illustrated in Scheme 54;[365] the related *threo*-



Reagents: i, NaN_3–DMF; ii, NaOMe–MeOH; iii, NaI–NaOAc–AcOH; iv, $POCl_3$–py

Scheme 54

diazide was obtained by performing the same series of reactions in the D-glucose series.

6′-Azido-6′-deoxymaltose has been prepared *via* a mono-*O*-toluene-*p*-sulphonylcyclohexa-amylose,[246] and syntheses of 6-azido- and 6,6′-diazido-derivatives of sucrose have been described.[248] 2-Azido-3,4-di-*O*-benzyl-2-deoxy-L-xylopyranose has been obtained from 3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-talofuranose by standard transformations.[366]

[363] A. S. Cerezo, *Anales Asoc. quim. argentina*, 1972, **60**, 355 (*Chem. Abs.*, 1973, **78**, 4455k).

[364] A. Piskorska-Chlebowska, *Roczniki Chem.*, 1973, **47**, 49.

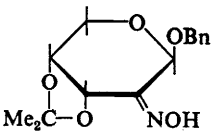
[365] J. Cleophax, D. Anglesio, S. D. Gero, and R. D. Guthrie, *Tetrahedron Letters*, 1973, 1769.

[366] H. Kuzuhara, H. Ohrui, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 349.

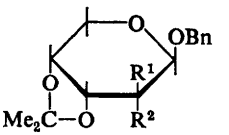
The reaction of sucrose octamethanesulphonate with azide ion in HMPT resulted in displacements at the 4,6,6′-positions (to give 6′-azido-6′-deoxy-β-D-fructofuranosyl 4,6-diazido-4,6-dideoxy-α-D-galactopyranoside pentamethanesulphonate) and at the 1′,4,6,6′-positions (to give 1′,6′-diazido-1′,6′-dideoxy-β-D-fructofuranosyl 4,6-diazido-4,6-dideoxy-α-D-galactopyranoside tetramethanesulphonate).[298]

Nitro-sugars

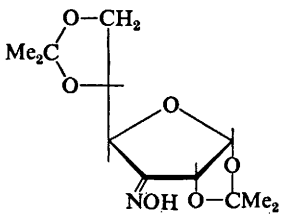
Several papers have appeared on the oxidation of sugar oximes with peroxy-acids; this provides a novel route to nitro-sugars by the reaction sequence $\rangle CH{-}OH \rightarrow \rangle C{=}O \rightarrow \rangle C{=}NOH \rightarrow \rangle CHNO_2$. Thus, oxidation of the oxime (142) with trifluoroperacetic acid furnished the epimeric nitro-sugars (143) and (144) in yields totalling 75%.[367] Similarly, peroxy-acid oxidation of (145) afforded (146), (147), and (148),[368] and that of (149) gave the 2-nitro-sugar (150);[369] transglycosylation occurred in alkaline methanol, presumably *via* the intermediacy of a nitro-olefin, to give the



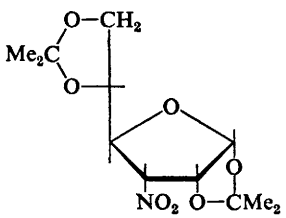
(142)



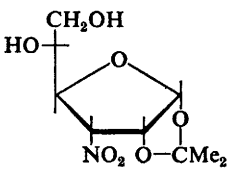
(143) $R^1 = H$; $R^2 = NO_2$
(144) $R^1 = NO_2$; $R^2 = H$



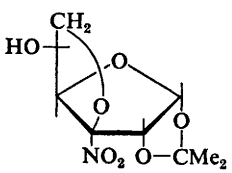
(145)



(146)

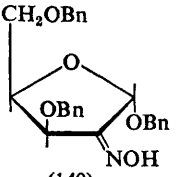


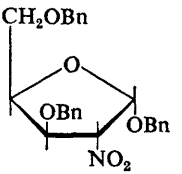
(147)



(148)

[367] T. Takamoto, M. Ohki, R. Sudoh, and T. Nakagawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 670.
[368] T. Takamoto, Y. Yokota, R. Sudoh, and T. Nakagawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 1532.
[369] T. Takamoto, R. Sudoh, and T. Nakagawa, *Carbohydrate Res.*, 1973, **27**, 135.

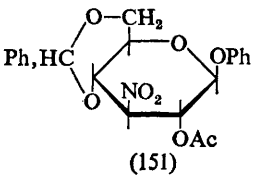

(149)

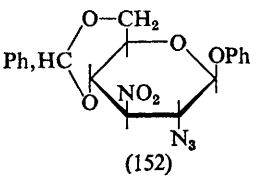

(150)

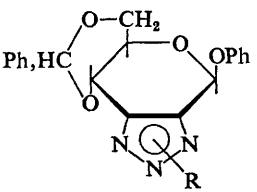
methyl glycoside of (150). Direct oxidation of amino-sugar derivatives with *m*-chloroperbenzoic acid also afforded nitro-sugars, together with nitroso-dimers.[370]

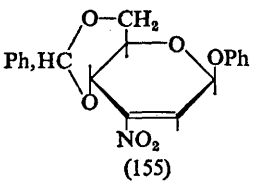
A derivative of 2,3-diamino-2,3-dideoxy-D-ribose has been prepared by way of a nitro-olefin[344] (see Scheme 50, p. 76), and the same type of intermediate has been used in syntheses of various branched-chain nitro-sugars[371] (see Chapter 15).

An extensive report has appeared on the formation of geminal halogenonitro-sugars[297] (see Scheme 41, p. 64). The reactions of a variety of amines (ethylamine, pyrrolidine, morpholine, *etc.*) with methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-α- and -β-D-glucopyranosides have been examined; the amine is introduced at C-2 by Michael addition to the nitro-olefin resulting from base-catalysed elimination of acetic acid.[372] The reaction of (151) with azide ion in basic solution furnished a mixture


(151)

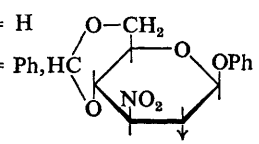

(152)




(155)

(153) R = H

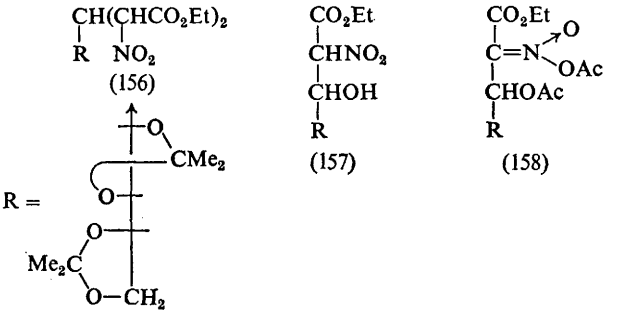
(154) R = Ph,HC



of (152), (153), and (154), presumably *via* the nitro-olefin (155), although the proportion of products could be varied by altering the basicity of the medium.[373]

[370] H. H. Baer and S.-H. Lee Chiu, *Canad. J. Chem.*, 1973, **51**, 1812.
[371] T. Sakakibara, T. Takamoto, R. Sudoh, and T. Nakagawa, *Chem. Letters*, 1972, 1219 (*Chem. Abs.*, 1973, **78**, 58 733t).
[372] F. J.-M. Rajabalee, *Carbohydrate Res.*, 1973, **26**, 219.
[373] T. Sakakibara, R. Sudoh, and T. Nakagawa, *J. Org. Chem.*, 1973, **38**, 2179.

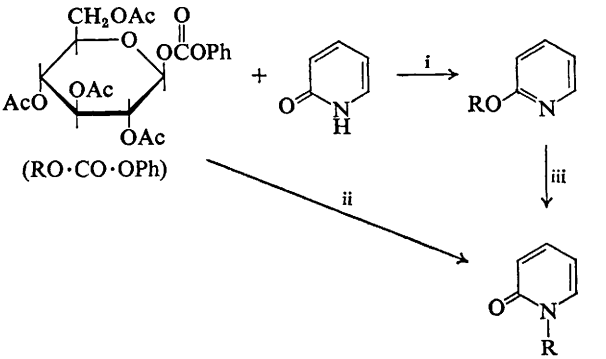
The condensation of *aldehydo*-sugars with ethyl nitroacetate has been investigated.[374] In the presence of amines, a 1:2-adduct, assigned the structure (156), was formed, whereas a 1:1-adduct (157) was obtained in the presence of ammonia. Acetylation of (157) with acetic anhydride in the presence of either acids or pyridine gave (158).



(156) (157) (158)

Heterocyclic Derivatives

The reaction of 2-pyridone with 2,3,4,6-tetra-*O*-acetyl-1-phenoxycarbonyl-β-D-glucopyranose in the presence of toluene-*p*-sulphonamide has been shown to yield *O*- or *N*-glycosyl derivatives, depending on the amount of catalyst employed[375] (Scheme 55). Hydrogenation of several *N*-(pent- or hex-2-enopyranosyl)benzotriazoles gave the corresponding 2,3-dideoxy-derivatives.[375a] A series of 1-aryl-3-alkyl(aryl)-4-(D-*arabino*-tetrahydroxybutyl)imidazoline-2-thiones (159) has been prepared by reaction of



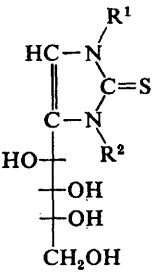
Reagents: i, $TsNH_2$ (1 eq.), 135—140 °C; ii, $TsNH_2$ (5 eq.), 135—140 °C; iii, $TsNH_2$ (5 eq.), 150 °C

Scheme 55

[374] V. I. Kornilov, B. B. Paidak, and Yu. A. Zhdanov, *J. Gen. Chem.* (*U.S.S.R.*), 1973, **43**, 185.

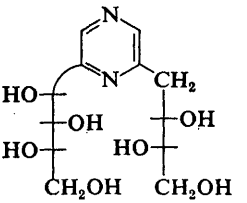
[375] M. Yamada, S. Inaba, T. Yoshino, and Y. Ishido, *Carbohydrate Res.*, 1973, **31**, 151.

[375a] M. Fuertes, G. Garcia-Muñoz, F. G. de las Heras, R. Madronero, and M. Stud, *J. Heterocyclic Chem.*, 1973, **10**, 503.

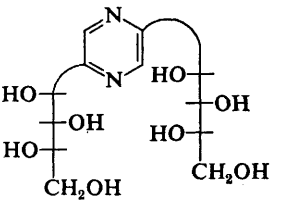


(159) R^1 = *p*-Me·C_6H_4,*p*-MeO·C_6H_4, or *p*-EtO·C_6H_4
R^2 = Me, Et, or Ph

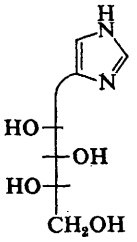
1-arylamino-1-deoxy-D-fructoses with alkyl(aryl) isothiocyanates.[376] Treatment of 1,3,4,5,6-penta-*O*-acetyl-*keto*-L-sorbose with ammonia gave products that included (160)—(163), as well as a number of simple heterocycles.[377] 1-Deoxy-1-(indol-1-yl)-D-galactitol and -glucitol have been prepared,[378] and D-ribose has been converted into 1-β-D-ribopyranosyl-5- and -6-fluoroindole.[379]



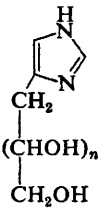
(160)



(161)



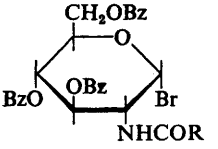
(162)



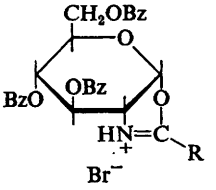
(163) n = 0, 1, or 2

[376] F. García Gonzáles, J. Fernandez-Bolaños, J. Fuentes Mota, and M. A. Pradera De Fuentes, *Carbohydrate Res.*, 1973, **26**, 427.
[377] M. C. Teglia and R. A. Cadenas, *Carbohydrate Res.*, 1973, **26**, 377.
[378] M. N. Preobrazhenskaya, V. I. Mukhanov, L. D. Manzon, and N. N. Suvorov, *Zhur. org. Khim.*, 1972, **8**, 2600.
[379] M. N. Preobrazhenskaya, V. I. Mukhanov, N. P. Kostuchenko, and N. N. Suvorov, *Zhur. org. Khim.*, 1973, **9**, 601.

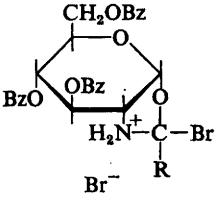
1,3-Dipolar additions of aromatic nitrile oxides to unsaturated sugars have been used to prepare isoxazolines.[380] A series of 2-acylamino-1,3,4,6-tetra-*O*-benzoyl-2-deoxy-α-D-glucopyranoses has been prepared, and their behaviour with either hydrogen bromide–acetic acid or hydrogen bromide–acetyl chloride was examined.[381] The products of such treatment were identified as 1-halogenoses (164), oxazolinium bromides (165), and



(164) R = Ph, Me_3C, *p*-$NO_2C_6H_4$, *etc.*



(165) R = *p*-MeO·C_6H_4 or *p*-BzNH·C_6H_4



(166) R = Me, $ClCH_2$, or Et

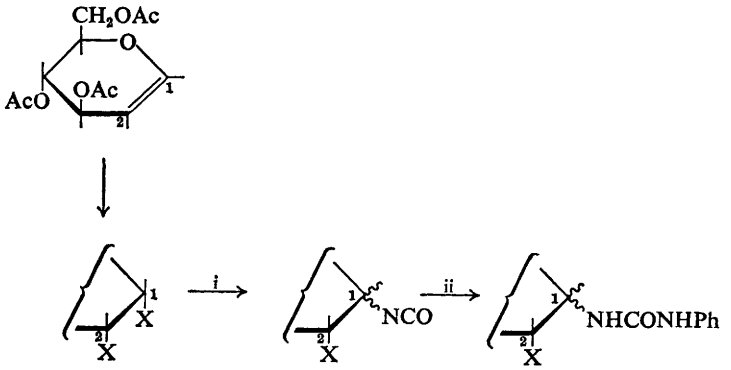
2-bromo-oxazolidinium bromides (166), depending on the *N*-acyl substituent. The isomerization of 2-methyl(phenyl)-(1′,2′-dideoxy-α-D-glucopyranosyl)[2′,1′:4,5]oxazolinium halides [*e.g.* (165)] to 2-acetamido-(benzamido)-2-deoxy-α-D-glucopyranosyl halides has been studied in solutions of both chloroform and acetone.[382]

Miscellaneous Compounds

cis-Addition of halogens to 3,4,6-tri-*O*-acetyl-D-glucal gave α-*gluco*- and β-*manno*-adducts, which were converted into 1-phenylureido-derivatives *via* the 1-isocyanate (see Scheme 56).[383]

Acetylation of D-aldose semicarbazones has been shown to give the cyclic (167) and acyclic (168) products, the proportion of which varied with the particular aldose used.[384]

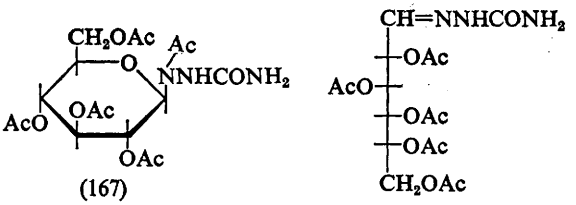
[380] J. M. J. Tronchet, S. Jaccard-Thorndahl, L. Faivre, and R. Massard, *Helv. Chim. Acta*, 1973, **56**, 1303.
[381] H. Weidmann, D. Tartler, P. Stöckl, L. Binder, and H. Hönig, *Carbohydrate Res.*, 1973, **29**, 135.
[382] H. Weidmann, P. Stöckl, D. Tartler, and H. Hönig, *Carbohydrate Res.*, 1973, **31**, 135.
[383] P. Boullanger, J.-C. Martin, and G. Descotes, *Bull. Soc. chim. France*, 1973, 2149.
[384] O. L. Galmarini, I. O. Mastronardi, and E. G. Gros, *Carbohydrate Res.*, 1973, **26**, 435.



X = halogen

Reagents: i, AgNCO; ii, $PhNH_2$

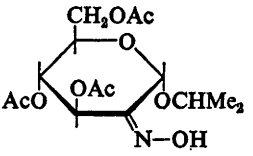
Scheme 56



N-Aryl-D-glucoheptosaminonitriles have been prepared by condensing D-glucose cyanohydrin with a series of aromatic bases.[385] Wohl degradation of octa-*O*-acetylmelibiononitrile has been reported to yield 1,1-(bisacetamido)-1-deoxy-5-*O*-α-D-galactopyranosyl-D-arabinitol, *N*-acetyl-5-*O*-α-D-galactopyranosyl-α-D-arabinofuranosylamine, and 5-*O*-α-D-galactopyranosyl-D-arabinofuranose.[386]

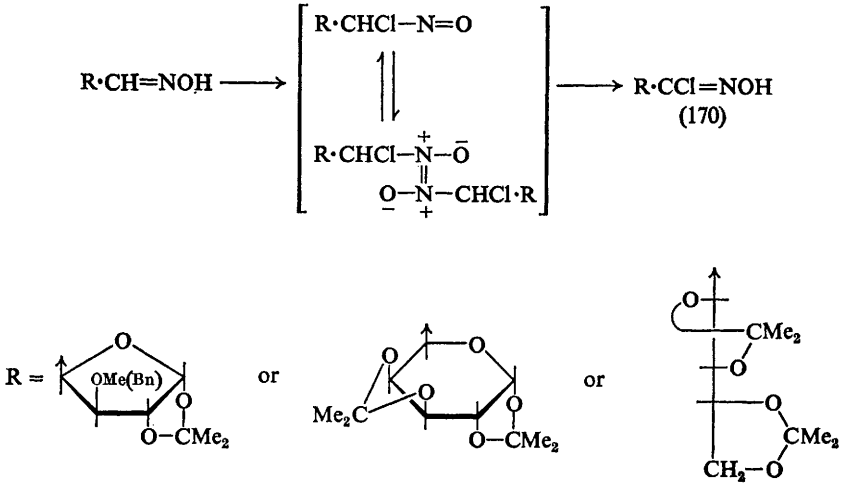
Detailed papers have appeared on the addition of nitrosyl chloride to acetylated glycals and on the chemistry of the adducts.[266, 267] Various methods for the reduction [305] and deoximation [387] of oximes (see Chapter 16) have been examined. The kinetics of the reactions of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride with simple alcohols in DMF to form alkyl 3,4,6-tri-*O*-acetyl-α-D-*arabino*-hexopyranosid-2-ulose oximes have been investigated by n.m.r. spectroscopy and polarimetry.[267] The oxime (169) has been demonstrated (n.m.r. evidence) to have the *syn*-configuration about the C=N bond.[387]

[385] H. Parekh, A. R. Parikh, and K. A. Thaker, *J. Indian Chem. Soc.*, 1972, **49**, 1147.
[386] J. O. Deferrari, B. N. Zuazo, and M. E. Gelpi, *Carbohydrate Res.*, 1973, **30**, 313.
[387] R. U. Lemieux, R. A. Earl, K. James, and T. L. Nagabhushan, *Canad. J. Chem.*, 1973, **51**, 19.

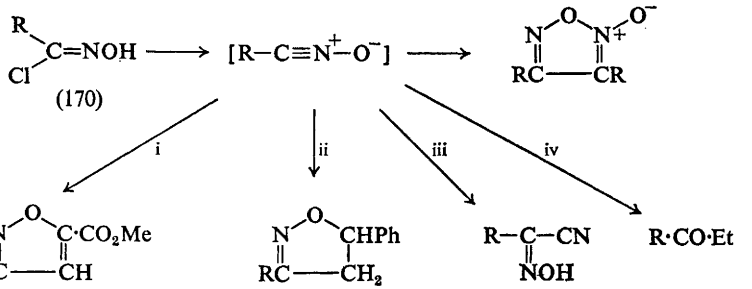


(169)

The chlorination of *aldehydo*-sugar oximes has been shown to take place *via* an S_E2' mechanism to give the corresponding *gem*-chloronitroso-derivatives, which are in equilibrium with their dimers[388] (Scheme 57). The



Scheme 57



Reagents: i, $HC{\equiv}C{\cdot}CO_2Me$; ii, $PhCH{=}CH_2$; iii, CN^-; iv, EtMgBr

Scheme 58

[388] J. M. J. Tronchet, F. Barbalat-Rey, N. Le-Hong, and U. Burger, *Carbohydrate Res.*, 1973, **29**, 297.

gem-chloronitroso-derivatives underwent isomerization into the hydroximoyl chlorides (170). Treatment of such hydroximoyl chlorides with base afforded unstable nitrile oxides, which, in the absence of nucleophilic or dipolarophilic reagents, dimerized to form furoxans.[389] The nitrile oxides are capable of undergoing a variety of 1,3-cycloadditions and other reactions (Scheme 58).

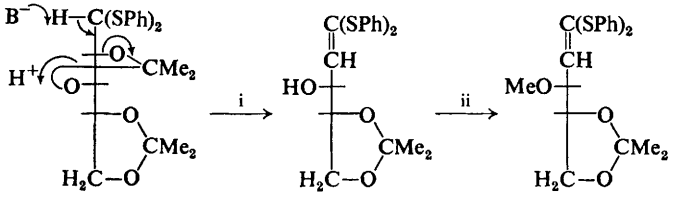
Both *O*- and *N*-saccharinyl glycosides of 2,3,4,6-tetra-*O*-methyl(acetyl)-D-glucopyranose have been described.[390]

[389] J. M. J. Tronchet and N. Le-Hong, *Carbohydrate Res.*, 1973, **29**, 311.
[390] A. Klemer and G. Uhlemann, *Annalen*, 1973, 1943.

11
Thio- and Seleno-sugars

Thio-sugars

Dithioacetals of 6-bromo- and 6-iodo-6-deoxy-D-galactose have been synthesized,[391] and trifluoroacetic acid has been used as catalyst in the formation of dithioacetals.[392] The unsaturated diphenyl dithioacetal (171) has been synthesized as shown in Scheme 59.[393] Both this compound and the



(171)

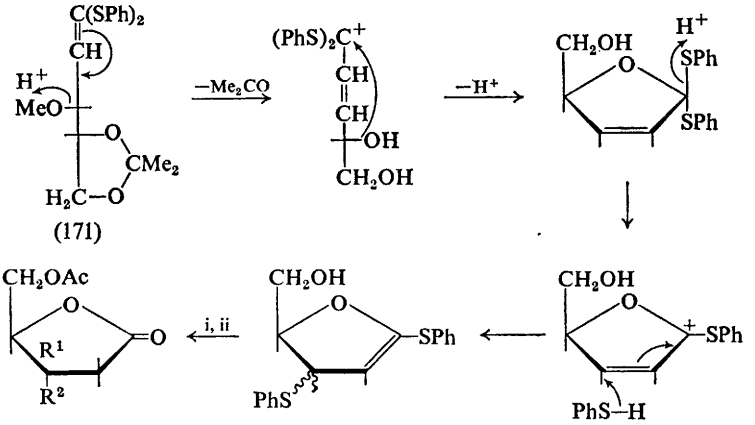
Reagents: i, Na–DMSO; ii, MeI

Scheme 59

related C-3 epimer (*cf.* Vol. 4, p. 80) were reported to be relatively unreactive towards reagents that usually attack alkenes or dithioacetals. With concentrated hydrochloric acid, they both gave, after acetylation, the lactones (172) and (173); a possible route to the products is shown in Scheme 60. 2-Lithio-1,3-dithian reacted with primary halogeno-groups to give *C*-(1′,3′-dithianyl) derivatives, which were converted into aldehydes and, thence, into primary alcohols [*e.g.* (174; R = CHO or CH_2OH)] as shown in Scheme 61.[394]

2-Thio-substituted sugars have been obtained in good yields by the photochemical addition of thiols to unsaturated sugars (Scheme 62).[395] Hardegger has reported a series of studies on the synthesis of derivatives

[391] J. Fernandez-Bolaños and R. Guzman de Fernandez-Bolaños, *Anales de Quim.*, 1973, **69**, 259.
[392] J. Fernandez-Bolaños and R. Guzman de Fernandez-Bolaños, *Anales de Quim.*, 1973, **69**, 263.
[393] B. Berrang, D. Horton, and J. D. Wander, *J. Org. Chem.*, 1973, **38**, 187.
[394] A. M. Sepulchre, G. Vass, and S. D. Gero, *Tetrahedron Letters*, 1973, 3619.
[395] Y. Araki, K. Matsuura, Y. Ishido, and K. Kushida, *Chem. Letters*, 1973, 383 (*Chem. Abs.*, 1973, **78**, 159 998p).

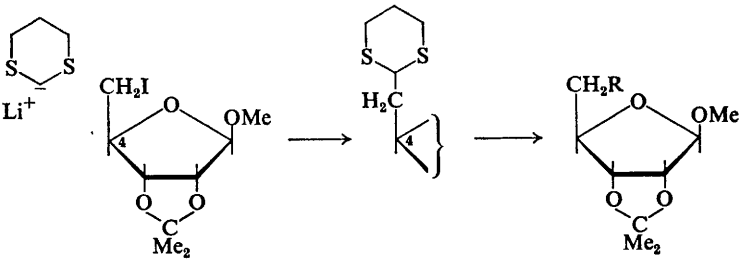


(171)

(172) R^1 = H, R^2 = SPh
(173) R^1 = SPh, R^2 = H

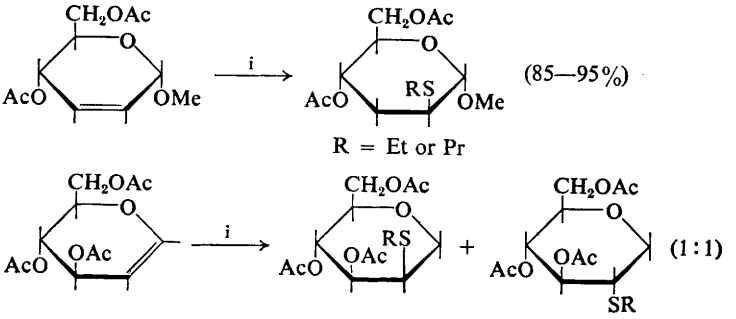
Reagents: i, H_2O; ii, Ac_2O

Scheme 60



(174)

Scheme 61

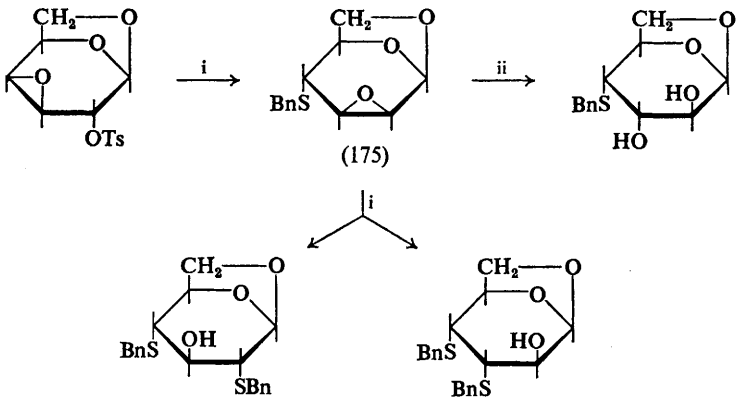


R = Et or Pr

Reagent: i, RSH–Me_2CO–$h\nu$

Scheme 62

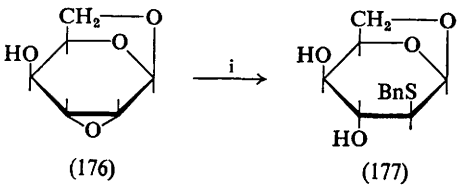
of 4-thio-D-glucopyranose, including the corresponding disulphide.[396] In the same paper, the synthesis of 1,6:2,3-dianhydro-4-*S*-benzyl-4-thio-β-D-mannopyranose (175) was described, as well as some ring-opening reactions of this epoxide, which apparently occur by both diaxial and diequatorial routes (Scheme 63). Diequatorial ring-opening was also reported to occur



Reagents: i, BnSNa–MeOH; ii, H^+

Scheme 63

in 1,6:2,3-dianhydro-β-D-gulopyranose (176) to give the 2-thio-D-idose derivative (177) (Scheme 64),[397] although this is contrary to previous experience with the dianhydride (N. R. Williams, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 109). However, the $J_{3,4}$ value of 4.5 Hz for the



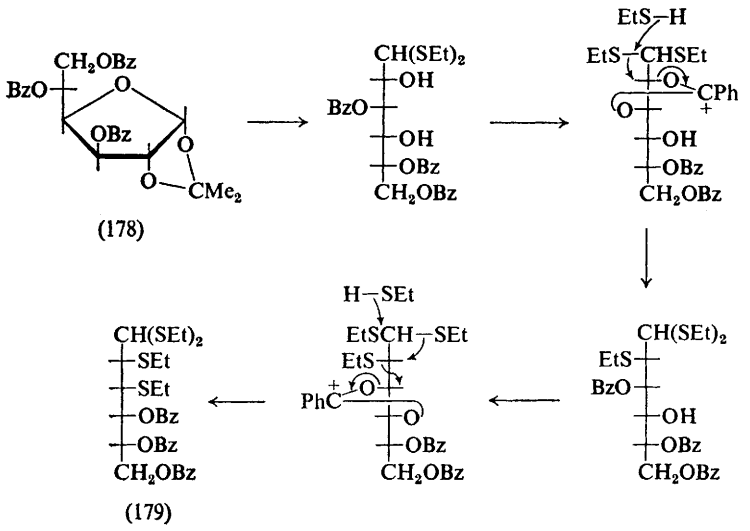
Reagent: i, BnSNa–MeOH

Scheme 64

derived diacetate is small for diaxially related protons at C-3 and C-4, so the structure assigned may be in error.

Bethell and Ferrier have produced evidence for the mechanism (Scheme 65) suggested for the formation of 4,5,6-tri-*O*-benzoyl-2,3-di-*S*-ethyl-2,3-dithio-D-allose diethyl dithioacetal (179) from 3,5,6-tri-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (178), by relayed transmission of ethyl-

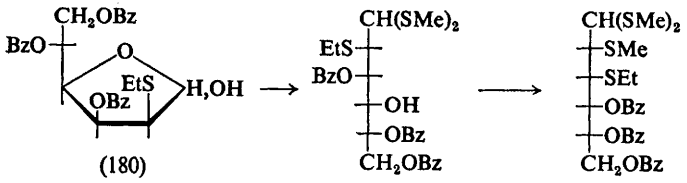
[396] L. Vegh and E. Hardegger, *Helv. Chim. Acta*, 1973, **56**, 1792.
[397] L. Vegh and E. Hardegger, *Helv. Chim. Acta*, 1973, **56**, 1961.



(178) (179)

Scheme 65

thio-groups along the carbon chain, in the presence of ethanethiol and hydrochloric acid (Vol. 6, p. 83).[398] Evidence for the involvement of 2-*S*-ethyl-2-thio-D-mannose intermediates was provided by the isolation of the trimethylthio-derivative (181) from methanethiolysis of the D-mannose derivative (180) (Scheme 66), while ethanethiolysis of the diacetate

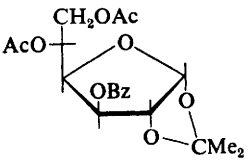


(180) (181)

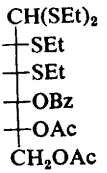
Scheme 66

3-benzoate (182), to give the 4-*O*-benzoyl-D-allose dithioacetal (183), indicated the involvement of the 3-*O*-benzoyl group in the reaction. The same authors have also reported that ethanethiolysis of 3-*O*-benzoyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (184) afforded ethyl 4-*O*-benzoyl-2,3,6-tri-*S*-ethyl-1,2,3,6-tetrathio-α-D-mannopyranoside (186).[399] The reaction was shown to follow a related route, the 3-*O*-benzoyl group now being involved in a third insertion of an ethylthio-group at C-6 *via* the benzoxonium ion (185) (Scheme 67).

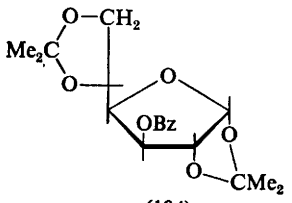
[398] G. S. Bethell and R. J. Ferrier, *J.C.S. Perkin I*, 1972, 2873.
[399] G. S. Bethell and R. J. Ferrier, *J.C.S. Perkin I*, 1973, 1400.



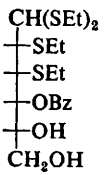
(182)

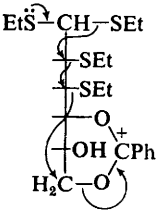


(183)

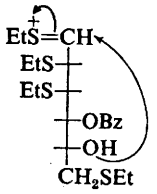


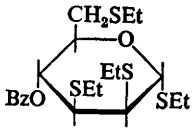
(184)





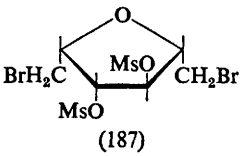
(185)



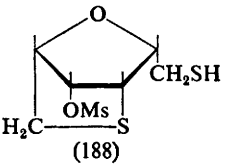


(186)

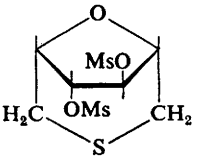
Scheme 67



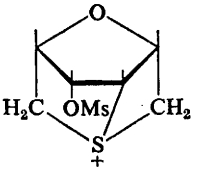
(187)



(188)



(189)



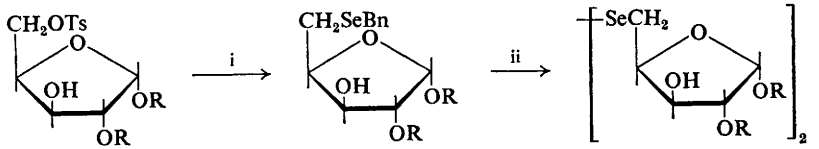
(190)

Starting with the dibromodimethanesulphonate (187), two types of bicyclic thioanhydrohexitol, (188) and (189), have been prepared.[400] Further displacements of the *exo*-methanesulphonate group in (189) proceeded either with retention of configuration or with ring contraction to give systems analogous to (188), suggesting the intermediacy of the sulphonium ion (190).

Other examples of thio-sugars are given in Chapter 12, and cyclic phosphates of 4′- and 5′-thioadenosine are reported in Chapter 21.

Seleno-sugars

Rabelo and Van Es have reported the preparation of derivatives of 5-seleno-D-xylofuranose. Displacements on the 5-sulphonates of methyl 2-*O*-methyl-α-D-xylofuranoside[401] and methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[402] were described, but the final products were invariably the diselenides (191) (see Scheme 68), rather than the required selenols,

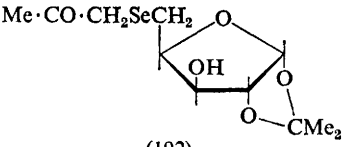


R, R = Me, Me; or $=CMe_2$ (191)

Reagents: i, BnSeNa–MeOH; ii, Na–NH_3

Scheme 68

thus forestalling any attempt at the eventual preparation of derivatives of 5-seleno-D-xylo- and -ribo-pyranoses. The diselenide (191) (R, R = $=CMe_2$) reacted with acetone in the presence of sulphuric acid and copper(II) sulphate to give the seleno-ether (192).[403] The same ether can be



(192)

obtained indirectly by cleavage of the diselenide with bromine, followed by treatment of the resulting selenoyl bromide with acetone in the presence of potassium thiocyanate.

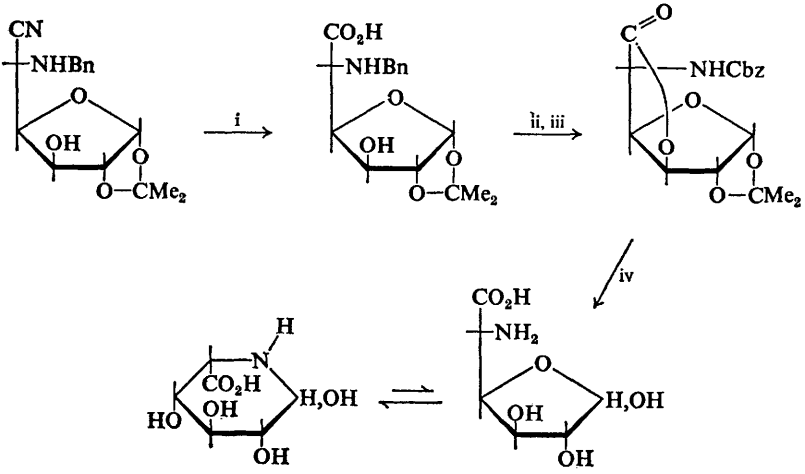
[400] J. Kuszmann and P. Sohár, *Carbohydrate Res.*, 1973, **27**, 157.
[401] J. J. Rabelo and T. Van Es, *Carbohydrate Res.*, 1973, **30**, 202.
[402] J. J. Rabelo and T. Van Es, *Carbohydrate Res.*, 1973, **30**, 381.
[403] T. Van Es and J. J. Rabelo, *Carbohydrate Res.*, 1973, **29**, 252.

12

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

Nitrogen Derivatives

5-Amino-5-deoxy-L-iduronic acid (related to the carbohydrate component of the polyoxins) has been synthesized as shown in Scheme 69.[404] The free



Reagents: i, H_2O; ii, H_2–Ni; iii, CbzCl; iv, H^+

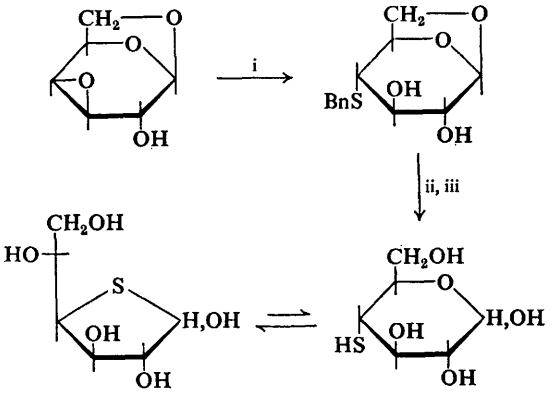
Scheme 69

acid was shown to exist as an equilibrium mixture of the furanose and pyranose forms, with the latter predominating; a number of derivatives of both forms were prepared.

Sulphur Derivatives

The main activity in this area has centred on D-glucose derivatives. 4-Thio-D-glucose has been synthesized,[405] and was shown to exist largely in

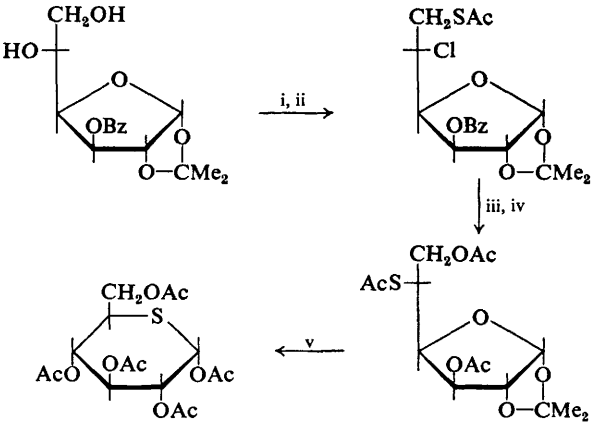
[404] H. Paulsen and E. Mäckel, *Chem. Ber.*, 1973, **106**, 1525.
[405] L. Vegh and E. Hardegger, *Helv. Chim. Acta*, 1973, **56**, 2020.



Reagents: i, BnSNa–MeOH; ii, Na–NH_3; iii, H^+

Scheme 70

the furanose form (see Scheme 70). Acetylation gave the furanose and pyranose penta-acetates in the ratio 7 : 3, and a number of other derivatives were described. Whistler's group has reported a new synthesis of 5-thio-α-D-glucopyranose penta-acetate involving the introduction of a sulphur grouping at C-6 and its transfer to C-5, as shown in Scheme 71.[406] 5-Thio-D-glucose has been studied as an inhibitor of the cellular-transport system of various compounds, including D-galactose, methyl α-D-glucopyranoside, and neutral amino-acids.[407] It also interfered with the transport of D-glucose, and is thus diabetogenic in character.

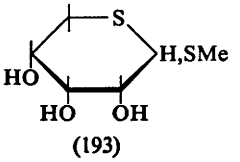


Reagents: i, Ph_3P–CCl_4; ii, KSAc; iii, KOH; iv, AcOH–Ac_2O–KOAc; v, AcOH–Ac_2O–H^+

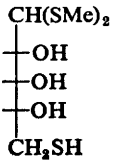
Scheme 71

[406] C.-W. Chiu and R. L. Whistler, *J. Org. Chem.*, 1973, **38**, 832.
[407] R. L. Whistler and W. C. Lake, *Biochem. J.*, 1972, **130**, 919.

Treatment of 5-thio-D-ribose with methanethiol in the presence of hydrochloric acid gave only methyl 1,5-dithio-α- and -β-D-ribopyranosides (193), and not the acyclic dithioacetal (194).[408] The dithioglycosides (193) were resistant to hydrolysis with acid and to methanolysis, but could be cleaved by acetolysis catalysed by either sulphuric acid or mercuric acetate.



(193)



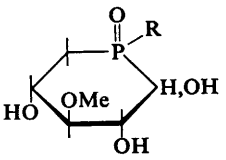
(194)

The preferred conformations of these and other 5-thio-D-ribopyranosides are discussed in Chapter 23.

Possible precursors of 5-seleno-D-xylo- and -D-ribo-pyranoses are mentioned in Chapter 11.

Phosphorus Derivatives

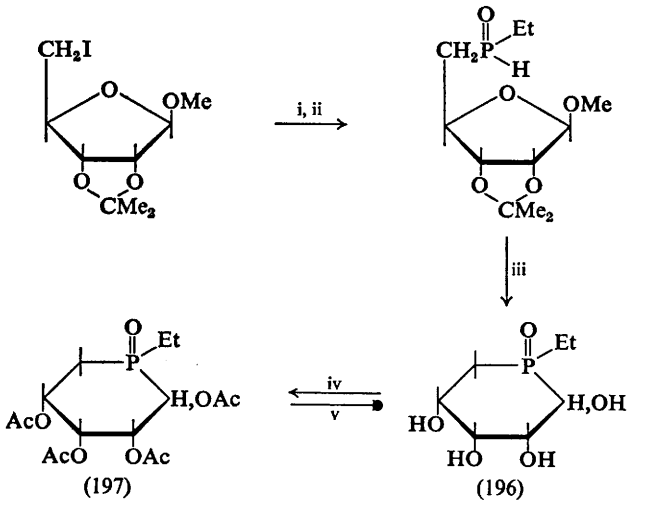
Inokawa's group have reported two more examples of this class of compound using the approach previously employed in the synthesis of the 5-deoxy-5-(ethylphosphonyl)-D-xylose derivative (*cf.* Vol. 5, p. 87). The first is simply a modification of an earlier synthesis, starting with the appropriately methylated precursor, to give the 3-*O*-methyl derivatives (195).[409] The second involved the D-ribose compound (196), the synthesis



(195) R = Et or Bu

of which is illustrated in Scheme 72; evidence for the pyranose structure (196) was derived from the absence of signals due to the PH group in the n.m.r. spectrum.[410] Attempts to form glycosides were unsuccessful, and treatment of (196) with acidified methanol left it unchanged. A syrupy tetra-acetate (197) was obtained, which reverted to the free sugar on Zemplen deacetylation.

[408] C. J. Clayton and N. A. Hughes, *Carbohydrate Res.*, 1973, **27**, 89.
[409] K. Seo and S. Inokawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 3301.
[410] S. Inokawa, H. Kitagawa, K. Seo, H. Yoshida, and T. Ogata, *Carbohydrate Res.*, 1973, **30**, 127.



(197) (196)

Reagents: i, $EtP(OEt)_2$; ii, $NaAlH_2(OCH_2CH_2OMe)_2$; iii, H^+; iv, Ac_2O; v, MeONa–MeOH

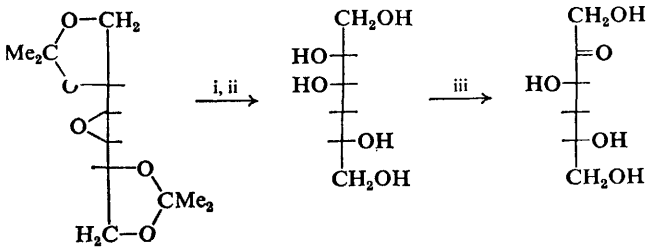
Scheme 72

13
Deoxy-sugars

Interest in the synthesis of deoxy-sugars from non-carbohydrate precursors has continued: 2-deoxy-DL-*erythro*-pentose [411, 412] and 2-deoxy-DL-*threo*-pentose [412] have been so prepared, and thioacetals and other derivatives of 3-deoxy-L-*threo*-pentose have been described.[413] Various racemic 4-deoxy-sugars have also been reported.[414]

3′-Deoxynucleosides are referred to in Chapter 21.

In the hexose series, 4-deoxy-D-*threo*-hexulose, the only previously unprepared deoxy-D-fructose, has been obtained by a process involving a specific, biochemical oxidation as the final step (see Scheme 73); other

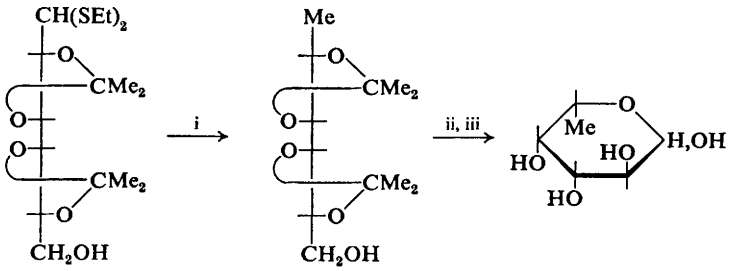


Reagents: i, $LiAlH_4$; ii, H^+; iii, *Acetobacter suboxydans*

Scheme 73

enzymic processes afforded the 6-phosphate and the 1,6-diphosphate of this sugar.[415] 4-Deoxy- and 4,6-dideoxy-derivatives of 2-acetamido-2-deoxy-D-glucose have been obtained using sulphuryl chloride, followed by reduction of the resulting chlorodeoxy-sugars with tributyltin hydride (see Vol. 6, p. 88),[416] and various deoxy-derivatives of 1,6-anhydro-β-D-hexopyranoses have also been prepared.[184]

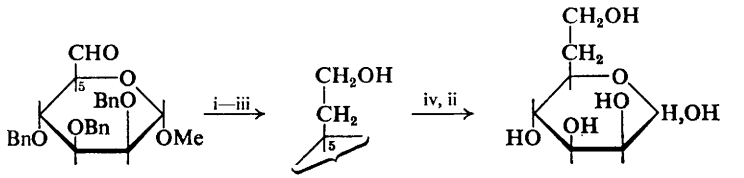
[411] M. Chmielewski and A. Zamojski, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1972, **20**, 751 (*Chem. Abs.*, 1973, **78**, 30 103p).
[412] O. A. Shavrygina, L. M. Kosheleva, and S. M. Makin, *Zhur. org. Khim.*, 1973, **9**, 74.
[413] H. Zinner and R. Reck, *J. prakt. Chem.*, 1973, **315**, 179.
[414] A. Banaszek and A. Zamojski, *Carbohydrate Res.*, 1972, **25**, 453.
[415] C. R. Haylock and K. N. Slessor, *Canad. J. Biochem.*, 1973, **51**, 969.
[416] H. Arita, K. Fukukawa, and Y. Matsushima, *Bull. Chem. Soc. Japan*, 1972, **45**, 3614.



Reagents: i, H_2–Ni; ii, DCC–DMSO; iii, H^+

Scheme 74

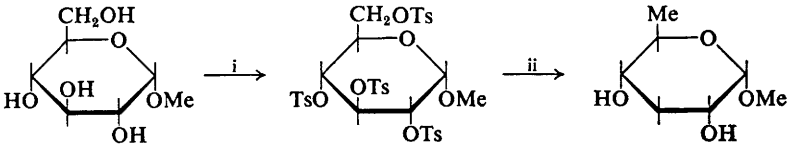
A convenient synthesis of L-fucose from D-galactose, the latter part of which is shown in Scheme 74, has been described,[417] and 6-deoxy-D-*manno*-heptose has been synthesized (Scheme 75) and shown to be identical with a constituent of a bacterial lipopolysaccharide.[418]



Reagents: i, $Ph_3P{=}CHOMe$; ii, H^+; iii, $NaBH_4$; iv, H_2–Pd

Scheme 75

In the dideoxyhexose series, methyl 3,6-dideoxy-α-D-*ribo*-hexopyranoside has been synthesized, in 16% overall yield, by a simple, two-step procedure from methyl α-D-glucopyranoside (Scheme 76).[419] It was proposed that a conventional reduction occurs at C-6, and that the C-2 ester (and subsequently the C-4 ester) is cleaved by S–O bond fission; a 2-*O*-linked aluminium complex then reductively displaces the toluene-*p*-sulphonyloxy-



Reagents: i, TsCl–py; ii, $LiAlH_4$

Scheme 76

[417] M. Dejter-Juszynski and H. M. Flowers, *Carbohydrate Res.*, 1973, **28**, 144.
[418] H. B. Boren, K. Eklind, P. J. Garegg, B. Lindberg, and A. Pilotti, *Acta Chem. Scand.*, 1972, **26**, 4143.
[419] G. Ekborg and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 1437.

group at C-3. In agreement with this proposal, lithium aluminium deuteride delivered a deuteride ion both at C-6 and C-3. A further report has appeared on 3,4-dideoxy-sugars,[420] and the hexulose intermediate in the bioconversion of D-glucose into 3,6-dideoxyhexoses is referred to in Chapter 16.

In the disaccharide series, the synthesis of methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-β-D-mannopyranoside has been mentioned already,[81] and 6-deoxy-,[293] 2,2′,3,3′-tetradeoxy-,[421] and 2,2′,3,3′,6,6′-hexadeoxy-αα-trehaloses [421] have been reported. In the course of their work with sucrose, Hough and Mufti have prepared 6- and 6′-deoxy-, and 6,6′-dideoxy-derivatives of the disaccharide.[298]

[420] S. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1972, **45**, 3619.
[421] A. C. Richardson and E. Tarelli, *J.C.S. Perkin I*, 1973, 1520.

14
Unsaturated Derivatives

A review (in Russian) has appeared on the synthesis of unsaturated sugars,[422] and a new, highly efficient reaction involving the use of phosphorus oxychloride in pyridine with iodo- or bromo-hydrins should have applications in this area.[423]

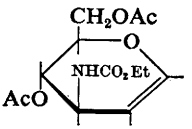
A number of aspects of unsaturated nucleosides are described in Chapter 21.

Glycals

Lemieux and his colleagues have published a series of eight papers detailing the use of glycal-nitrosyl chloride additions in the synthesis of glycosidic products.[89–91, 266, 267, 305, 387, 424]

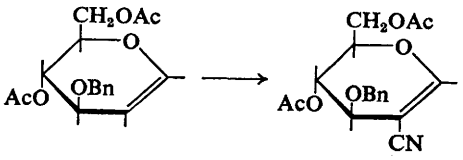
Optimum conditions for the methoxymercuration of 3,4,6-tri-*O*-acetyl-D-glucal have been described, and oxymercuration in the presence of partially protected sugars has led to the synthesis of 2-deoxy-D-*arabino*-hexosyl disaccharides.[425] Similar products can be obtained by using collidine, silver perchlorate, and iodine in the initial addition step.[426] The oxidation of 3,4,6-tri-*O*-acetyl-D-glucal to 2-deoxyglyconic esters is referred to in Chapter 17.

Jordaan and his co-workers have published details of a reaction by which the glycal derivative (198) can be obtained from a 2,3-unsaturated glycoside (*cf.* Vol. 5, p. 91),[427] and have shown that 2-cyanoglycals can be prepared using chlorosulphonyl isocyanate, followed by triethylamine



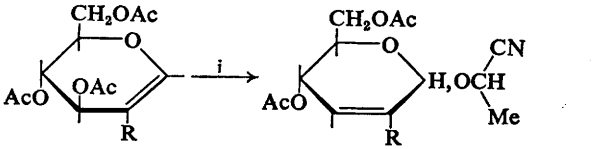
(198)

[422] Yu. A. Zhdanov and V. G. Alekseeva, *Uspekhi khim.*, 1973, **42**, 1085.
[423] A. Guzmán, P. Ortiz de Montellano, and P. Crabbé, *J.C.S. Perkin I*, 1973, 91.
[424] R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson, and L. C. N. Tucker, *Canad. J. Chem.*, 1973, **51**, 53.
[425] S. Honda, K. Kakehi, H. Takai, and K. Takiura, *Carbohydrate Res.*, 1973, **29**, 477.
[426] S. Honda, K. Kakehi, and K. Takiura, *Carbohydrate Res.*, 1973, **29**, 488.
[427] R. H. Hall, A. Jordaan, and G. J. Lourens, *J.C.S. Perkin I*, 1973, 38.



Scheme 77

(Scheme 77).[428] Japanese workers have studied the photochemical addition of acetone to 3,4,6-tri-*O*-acetyl-D-glucal (*cf.* Vol. 6, p. 92), and found that the concentration of acetone has an important bearing on the type of product formed.[429, 430] Photochemical addition of acetaldehyde cyanohydrin to acetylated D-glucal and 2-hydroxy-D-glucal afforded 2-cyanoethyl glycosides, and it was also shown that 2,3-unsaturated sugars can be obtained from these reactions (Scheme 78).[93] *cis*-Addition of halogens to



R = H; α-anomer 37%, β-anomer 47%
R = OAc; α-anomer 40%, β-anomer 40%

Reagent: i, MeCH(OH)CN–*hν*

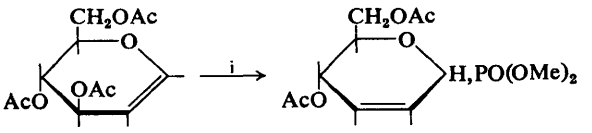
Scheme 78

3,4,6-tri-*O*-acetyl-D-glucal furnished α-*gluco*- and β-*manno*-adducts, which were subsequently transformed into glycosyl isocyanates (see Scheme 56).[383]

The well-known 1-ene to 2-ene rearrangement has been further studied in the reaction between 3,4,6-tri-*O*-acetyl-D-glucal and purine derivatives.[431] As noted by several groups, anomeric pairs of 2,3-unsaturated nucleosides are produced, together with glycal derivatives having the purines bonded at C-3. The products were characterized by chemical and ^{1}H n.m.r. spectroscopic methods, and were discussed in relation to various antibiotic substances.

The same kind of rearrangement occurred in the reaction between 3,4,6-tri-*O*-acetyl-D-glycals and dimethyl phosphite in the presence of boron trifluoride (Scheme 79); since D-allal and D-glucal derivatives gave

[428] R. H. Hall and A. Jordaan, *J.C.S. Perkin I*, 1973, 1059.
[429] K. Matsuura, Y. Araki, and Y. Ishido, *Bull. Chem. Soc. Japan*, 1972, **45**, 3496.
[430] K. Matsuura, Y. Araki, Y. Ishido, A. Murai, and K. Kushida, *Carbohydrate Res.*, 1973, **29**, 459.
[431] E. E. Leutzinger, T. Meguro, L. B. Townsend, D. A. Shuman, M. P. Schweizer, C. M. Stewart, and R. K. Robins, *J. Org. Chem.*, 1972, **37**, 3695.

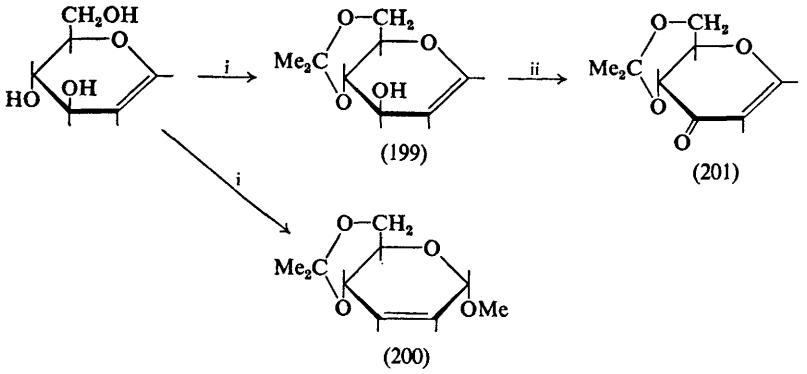


Reagent: i, $HPO(OMe)_2$–BF_3

Scheme 79

the same anomeric ratio ($\alpha : \beta$, 1 : 2) of phosphonates, it was inferred that the reaction proceeds by a unimolecular process involving pre-ionization.[432] For this conclusion to be valid, it is necessary that the products do not interconvert after formation. This type of allylic rearrangement has also been used in the synthesis of a dideoxy-derivative of a disaccharide.[92]

Acetonation of D-glucal has been shown to give initially the 4,6-*O*-isopropylidene derivative (199) but, if long reaction times are used, the proportion of the 2-ene (200) is increased (Scheme 80). The first-formed

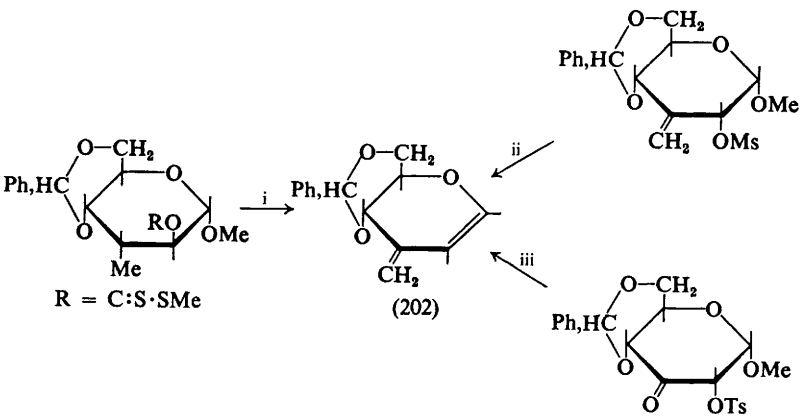


Reagents: i, $Me_2C(OMe)_2$–DMF–TsOH; ii, MnO_2 or CrO_3–py

Scheme 80

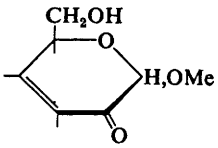
product has been employed in a synthesis of the enone (201).[433] Work on a related enone is mentioned in Chapter 16. *C*-Methylenation (by a Wittig reaction) of the benzylidene analogue of the enone (201) furnished the conjugated diene (202), which was also prepared by three other routes (Scheme 81).[434] Convenient routes to methyl 3,4-dideoxy-D-*glycero*-hex-3-enopyranosid-2-ulose (203) have been developed for use on a large scale; these have depended chiefly on reductive elimination of either vicinal

[432] H. Paulsen and J. Thiem, *Chem. Ber.*, 1973, **106**, 3850.
[433] B. Fraser-Reid, D. L. Walker, S. Y.-K. Tam, and N. L. Holder, *Canad. J. Chem.*, 1973, **51**, 3950.
[434] S. Y.-K. Tam, D. E. Iley, N. L. Holder, D. R. Hicks, and B. Fraser-Reid, *Canad. J. Chem.*, 1973, **51**, 3150.



Reagents: i, heat; ii, LiI–Et_2O; iii, CH_2I_2–Mg

Scheme 81



(203)

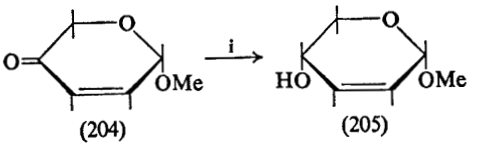
cis- or *trans*-disulphonyloxy-groups.[435] Contrary to earlier reports, it was demonstrated that the allylic hydroxy-groups of these compounds are oxidized with manganese dioxide, irrespective of whether they adopt pseudo-axial or -equatorial orientations.

Photoaddition of thiols to 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal gave 1-thioglycosides in good yield, although non-specific addition occurred at C-2 of 3,4,6-tri-*O*-acetyl-D-glucal to give derivatives of 1,5-anhydro-D-glucose (see Scheme 62).[395]

Other Unsaturated Compounds

2-Enoses have received a good deal of attention; a number of reports have dealt with their synthesis from achiral precursors and further transformations thereon. Several chiral 2-enoses are noted in the section on glycals. Reduction of the enone (204) with lithium aluminium hydride gave the 2-enoside (205) with 90% stereoselectivity (Scheme 82).[436] All the 3-enosides have been prepared from the 2-enoside (206) as shown in Scheme 83;[414] the conversion of these epoxides directly into allylic alcohols with butyl-lithium was successful only in the case of methyl 2,3-anhydro-4-deoxy-α-DL-*lyxo*-hexopyranoside (Scheme 84).

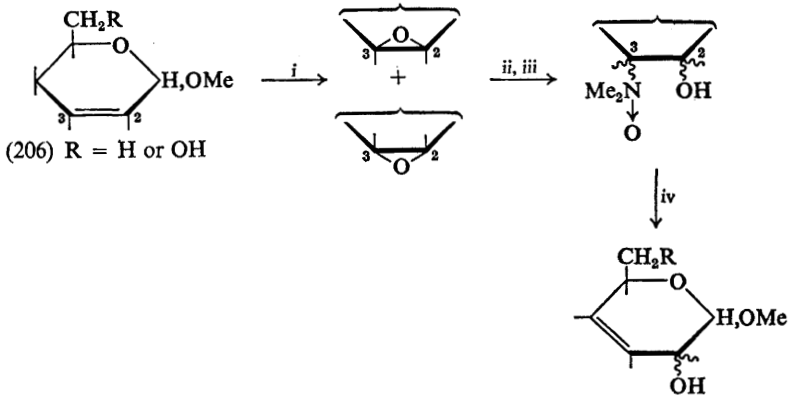
[435] N. L. Holder and B. Fraser-Reid, *Canad. J. Chem.*, 1973, **51**, 3357.
[436] O. Achmatowicz, jun., and P. Bukowski, *Roczniki Chem.*, 1973, **47**, 99.



(204) (205)

Reagent: i, $LiAlH_4$

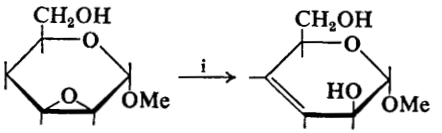
Scheme 82



(206) R = H or OH

Reagents: i, peracid; ii, Me_2NH–H_2O; iii, H_2O_2; iv, heat

Scheme 83



Reagent: i, BuLi

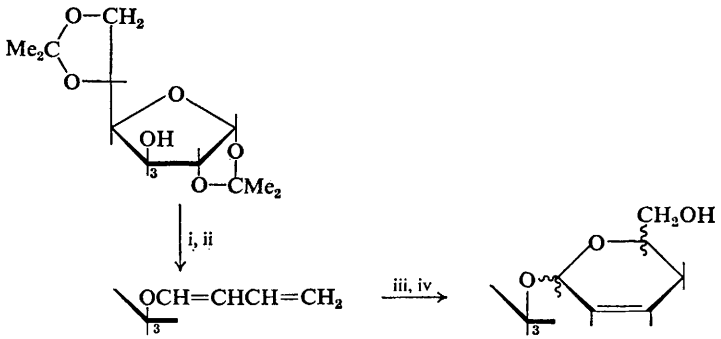
Scheme 84

Reference to the synthesis of a derivative of 4-amino-2,3,4,6-tetradeoxy-L-*erythro*-hexose (tolyposamine) from a 2,3-unsaturated glycoside is made in Chapter 8 (Scheme 47).[322]

A novel method for synthesizing disaccharides and their derivatives has been based on building up an unsaturated glycosyl moiety by way of a dienic ether attached to a partially protected sugar derivative (see Scheme 85).[437]

^1H N.m.r. analyses of the conformations of 2,5-dimethyl-5,6-dihydro-α-pyrans should provide useful comparisons with those of 2- and 3-eno-pyranosides.[438]

[437] S. David, J. Eustache, and A. Lubineau, *Compt. rend.*, 1973, **276**, *C*, 1465.
[438] K. Jankowski and J. Couturier, *J. Org. Chem.*, 1972, **37**, 3997.



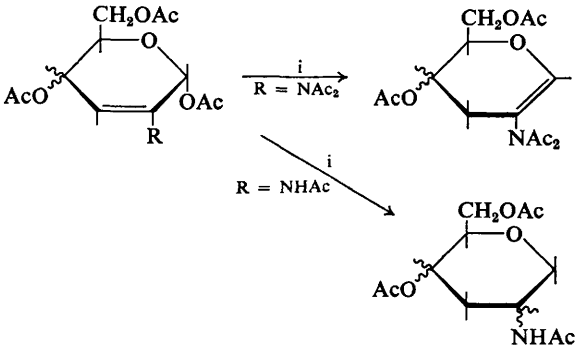
Reagents: i, $Me_2C(OH)C{\equiv}CC{\equiv}CC(OH)Me_2$–THF–KOH; ii, H_2–Pd–$BaSO_4$; iii, $BuCO_2{\cdot}CHO$; iv, $LiAlH_4$

Scheme 85

Unsaturated derivatives of 1,6-anhydro-β-D-hexopyranoses have been used in the preparation of related deoxy-derivatives.[185]

The use of 3-*O*-vinyl-hex-2-enopyranosides in the synthesis of branched-chain sugars is mentioned in Chapter 15.

Compounds having 2-enopyranose structures and carrying substituents at the vinylic positions have been reported. Further work on the preparation[439] and hydrogenation[309] of 2-acetamido- and *NN*-diacetylamino-sugar derivatives has been carried out by Fletcher's group (*cf.* Vol. 5, p. 94), a new feature being the unexpected formation of a glycal (Scheme 86).[309] Elimination occurred on acetylation of 4,6-*O*-benzylidene-2-benzyloxycarbonylamino-2-deoxy-D-gluconic acid to give the enamine derivative (207).[440]

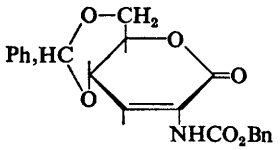


Reagents: i, H_2–Pd–AcOH

Scheme 86

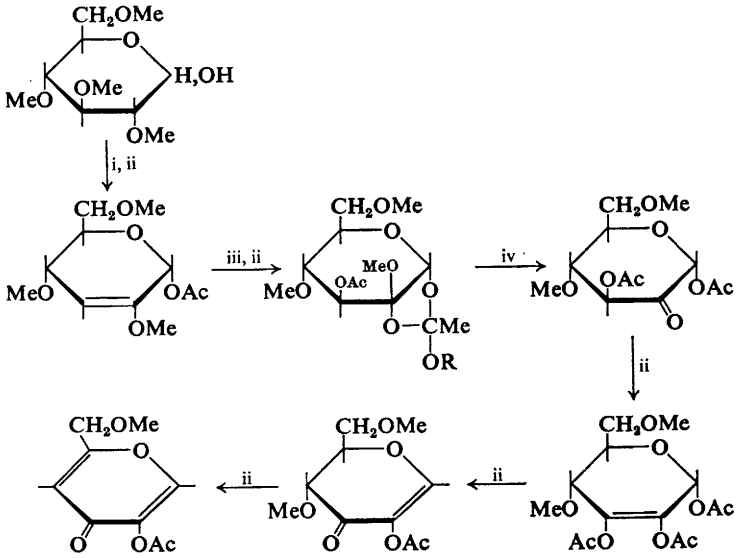
[439] N. Pravdic, B. Zidovec, and H. G. Fletcher, jun., *Croat. Chem. Acta*, 1973, **45**, 333 (*Chem. Abs.*, 1973, **79**, 79 092u).

[440] G. Deak, K. Gall-Istok, and P. Sohar, *Acta Chim. Acad. Sci. Hung.*, 1973, **75**, 189.



(207)

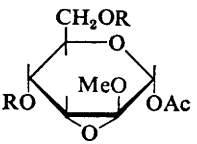
As part of a continuing study of the base-catalysed degradation of carbohydrates, the sequence of reactions represented in Scheme 87 has been carried out.[178] A minor product of peroxy-acid oxidation was shown to be the epoxide derivative (208); however, the triacetate (209) yielded the



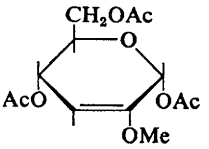
Reagents: i, HO^-; ii, Ac_2O–py; iii, *m*-$ClC_6H_4CO_3H$; iv, Zn–AcOH

Scheme 87

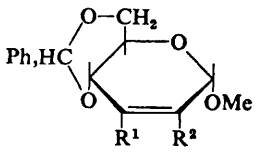
epoxide (210) as the major product, with a 1,2-orthoester appearing as the minor product. The latter orthoester was converted into 2-ulose derivatives and D-ribono-1,4-lactone triacetate.[178a]



(208) R = Me
(210) R = Ac

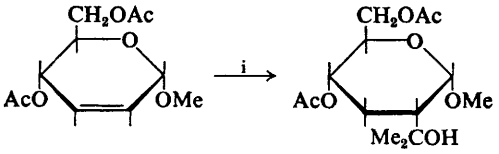


(209)



(211) R^1 = H; R^2 = Me
(212) R^1 = Me; R^2 = H

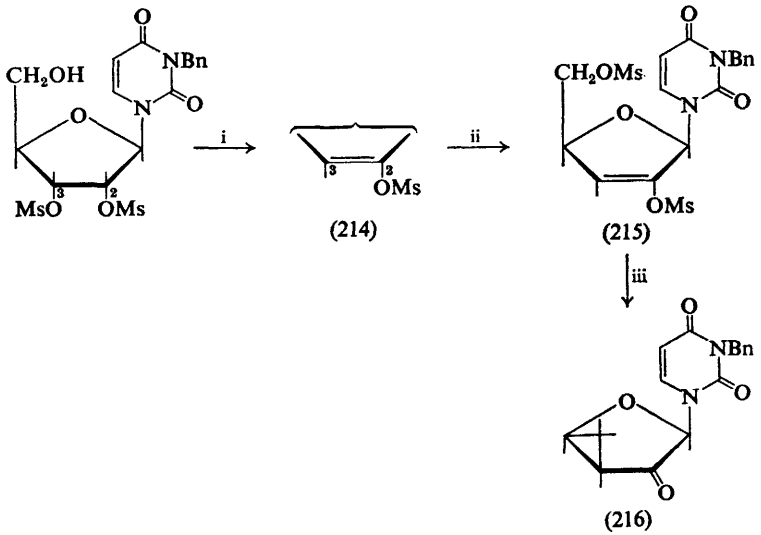
The branched-chain derivatives (211) and (212), having branching at the vinylic positions, have been obtained by Chugaev eliminations on appropriate xanthates of 2- and 3-*C*-methylpyranosides.[177] Related products [*e.g.* (213)] were obtained by photochemically induced additions of secondary alcohols to unsaturated sugars (Scheme 88),[441, 442] and the photoaddition of thiols to a 2,3-dideoxyhex-2-enoside is described above.[395]



(213)

Reagent: i, Me_2CHOH–*hν*

Scheme 88

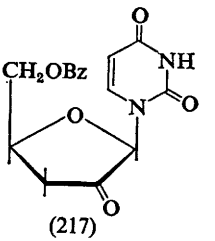


(214) (215) (216)

Reagents: i, NaOBz–DMF; ii, MsCl–py; iii, K_2CO_3–DMF–MeCN

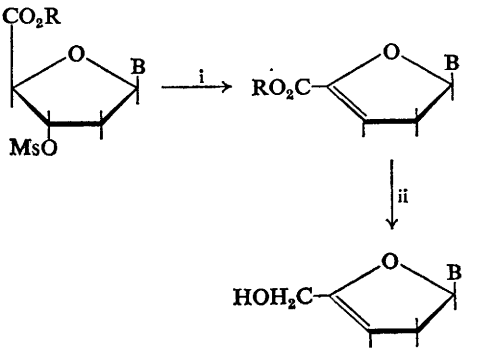
Scheme 89

In the area of pent-2-enofuranosides, several reports of relevance to nucleoside chemistry have appeared. The reaction of methyl 5-*O*-benzoyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranoside with bromine in the presence of methanol and silver acetate is noted in Chapter 7.[295] Selective base-catalysed elimination of sulphonic acid from 2′,3′-di-*O*-sulphonylnucleosides gave 3′-deoxy-pent-2′-enosyl products in the cases of purine toluene-*p*-sulphonates[443] and uridine methanesulphonates (Scheme 89).[444] Mesylation of (214) gave the disulphonate (215), which afforded the interesting cyclopropyl derivative (216) on treatment with potassium carbonate in aprotic solvents; a number of reactions and compounds related to those shown in this Scheme were reported. Analogous elimination of the 3′-sulphonyloxy-group occurred with β-D-lyxosylnucleoside disulphonates, and a 2-keto-nucleoside (217) was obtained from the resulting enol ester.[445]



(217)

Elimination of hydrogen bromide from a 2′-bromo-2′-deoxyuridine derivative,[296] and an unsaturated derivative of sucrose[292] are referred to in Chapter 7.

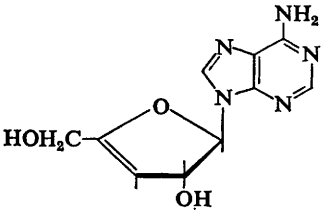


Reagents: i, Et_3N; ii, $NaAl(OCH_2CH_2OMe)_2H_2$

Scheme 90

[441] K. Matsuura, Y. Araki, Y. Ishido, and S. Satoh, *Chem. Letters*, 1972, 849 (*Chem. Abs.*, 1973, **78**, 30 104q).
[443] K. Matsuura, Y. Araki, Y. Ishido, and M. Kainosho, *Chem. Letters*, 1972, 853.
[443] T. Sasaki, K. Minamoto, and S. Tanizawa, *J. Org. Chem.*, 1973, **38**, 2896.
[444] T. Sasaki, K. Minamoto, and H. Suzuki, *J. Org. Chem.*, 1973, **38**, 598.
[445] T. Sasaki, K. Minamoto, and K. Hattori, *J. Org. Chem.*, 1973, **38**, 1283.

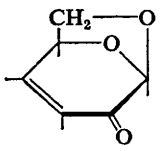
A full paper on 3,4-unsaturated sugars and dideoxy-derivatives thereof has appeared,[420] and a good survey of unsaturated glycofuranosylnucleosides precedes the description of a method for preparing 2′,3′-dideoxy-glyc-3′-enofuranosyl compounds (Scheme 90).[446] A related 3′,4′-dideoxypent-3′-enosyl nucleoside (218) has been obtained in the course of work with 3′-deoxy-3′-halogeno-nucleosides.[179]



(218)

Studies, carried out mainly in Poland, have been devoted to syntheses of carbohydrate derivatives from achiral precursors. Both anomers of racemic methyl 3,4-dideoxy-pent-3-enopyranoside have been prepared from 5,6-dihydro-2-methoxypyran [447] and they were used to obtain the eight stereoisomeric methyl pentopyranosides.[448] Bishydroxylations of methyl 3,4-dideoxy-hex-3-enopyranosides have been studied in related work.[449]

One of the main components of a tar formed on pyrolysis of cellulose, D-glucose, or 1,6-anhydro-β-D-glucopyranose has been identified as the enone (219), and structures previously assigned to this compound appear to be in error.[450]

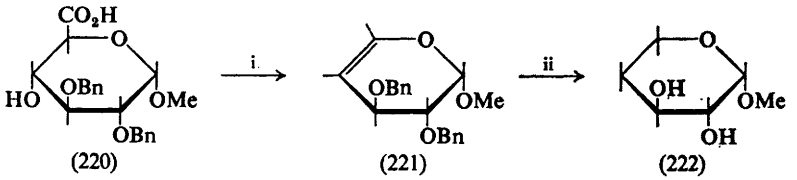


(219)

Other 3,4-unsaturated furanosyl compounds are referred to in Chapters 6 and 15, and a diene prepared from sucrose is mentioned in Chapter 6.

The uronic acid derivative (220) has been shown to undergo eliminative decarboxylation to furnish the pent-4-enoside (221) in high yield; hydrogenation and debenzylation then gave methyl 4-deoxy-β-L-*threo*-pentopyranoside (222) (Scheme 91).[451] Eliminations from other uronosides are referred to in Chapter 17.

[446] J. Žemlička, J. V. Friesler, R. Gasser, and J. P. Horwitz, *J. Org. Chem.*, 1973, **38**, 990.
[447] M. Chmielewski and A. Zamojski, *Roczniki Chem.*, 1972, **46**, 1767.
[448] M. Chmielewski and A. Zamojski, *Roczniki Chem.*, 1972, **46**, 2223.
[449] A. Banaszek, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1972, **20**, 925 (*Chem. Abs.*, 1973, **78**, 30 144c).
[450] Y. Halpern, R. Riffer, and A. Broido, *J. Org. Chem.*, 1973, **38**, 204.
[451] K. D. Philips, J. Žemlička, and J. P. Horwitz, *Carbohydrate Res.*, 1973, **30**, 281.



(220) (221) (222)

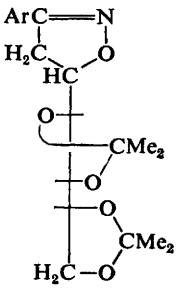
Reagents: i, $Me_2NCH(OCH_2CMe_3)_2$–heat; ii, H_2–Pd–C

Scheme 91

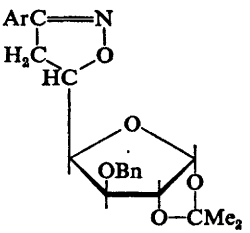
Interest has continued in applications of the Wittig and related reactions in carbohydrate chemistry; applications to terminal carbonyl groups are noted here, whereas branched-chain sugars formed from non-terminal carbonyl derivatives are mentioned in Chapters 8, 15, and 16. Tronchet and his colleagues have published extensively on Wittig reactions applied to 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-pentodialdo-1,4-furanose, and have described a variety of olefinic products.[452–454] 2,5-Anhydro-3,4-*O*-isopropylidene-D-ribose has been used to prepare derivatives of *C*-glycosides.[452]

Related work with acyclic, aldehydic compounds, which allows extension of the carbon chain of aldoses, has continued.[393, 455, 456] Olefinic compounds prepared by these methods gave such isoxazolines as (223) and (224) on reaction with aromatic nitrile oxides.[380]

The reactions of a nitro-olefinic sugar are referred to in Chapter 19.



(223)



(224)

Ar = *p*-$NO_2 \cdot C_6H_4$ or 2,4,6-$Me_3 \cdot C_6H_2$

[452] J. M. J. Tronchet, B. Baehler, H. Eder, N. Le-Hong, F. Perret, J. Poncet, and J.-B. Zumwald, *Helv. Chim. Acta*, 1973, **56**, 1310.

[453] J. M. J. Tronchet, C. Cottet, B. Gentile, E. Mihaly, and J.-B. Zumwald, *Helv. Chim. Acta*, 1973, **56**, 1802.

[454] J. M. J. Tronchet, M.-Th. Campanini, J. Denoyelle, and J.-B. Zumwald, *Helv. Chim. Acta*, 1973, **56**, 2567.

[455] D. Horton, A. Liav, and S. E. Walker, *Carbohydrate Res.*, 1973, **28**, 201.

[456] H. Ogura, M. Ogiwara, T. Itoh, and H. Takahashi, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 2051.

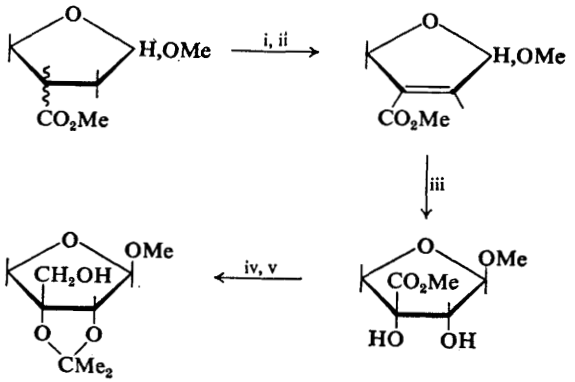
15
Branched-chain Sugars

As in previous Volumes, this Chapter is divided into two sections depending on the type of branching present. However, one paper has described a simple synthesis of branched-chain sugar derivatives that effectively contain both $R^1—C—OR^2$ and $R—C—H$ branches. These compounds resulted from the photochemical addition of secondary alcohols to unsaturated glycosides, an example of which is shown in Scheme 88; photoaddition of primary alcohols was less successful.[441]

Compounds with an $R^1—C—OR^2$ Branch

Chapter 16 contains examples of the reactions of alduloses with such nucleophiles as diazomethane, nitromethane, and ethyl zincbromoacetate, which lead eventually to the synthesis of branched-chain sugars of the $R^1—C—OR^2$ type. The Reformatski reaction has also been used to prepare branched-chain 2-deoxyaldonic acids.[457]

A derivative of DL-apiose has been synthesized from 2-methoxy-4-methoxycarbonyltetrahydrofuran as shown in Scheme 92,[458] and an



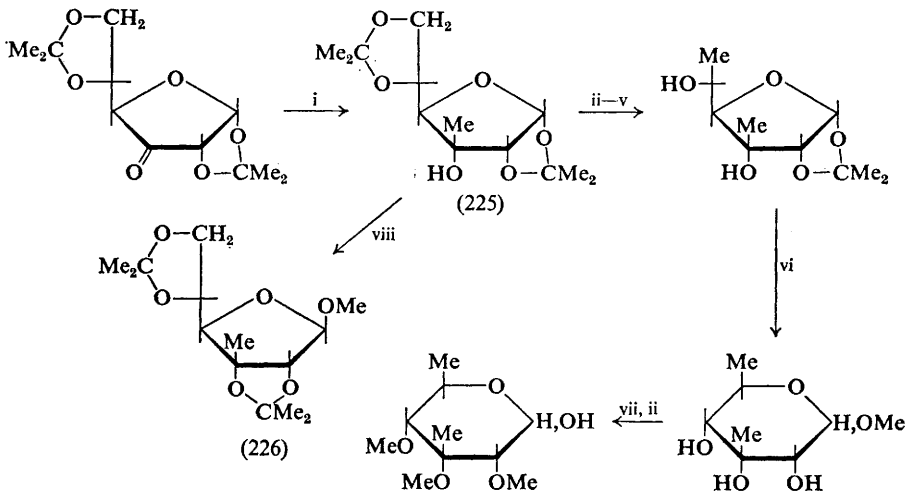
Reagents: i, $C_5H_5N·HBr_3$; ii, DBU–PhH; iii, OsO_4; iv, $Me_2CO–H^+$; v, $LiAlH_4$

Scheme 92

[457] Yu. A. Zhdanov, Yu. E. Alekseev, and Kh. A. Kurdanov, *J. Gen. Chem.* (*U.S.S.R.*), 1973, **43**, 182.

[458] T. Kinoshita and T. Miwa, *Carbohydrate Res.*, 1973, **28**, 175.

enzymic synthesis of UDP-D-apiose from UDP-D-glucuronic acid has been reported.[459] A new route to 6-deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-D-allopyranose (227) is depicted in Scheme 93; the D-*allo*-configuration of the



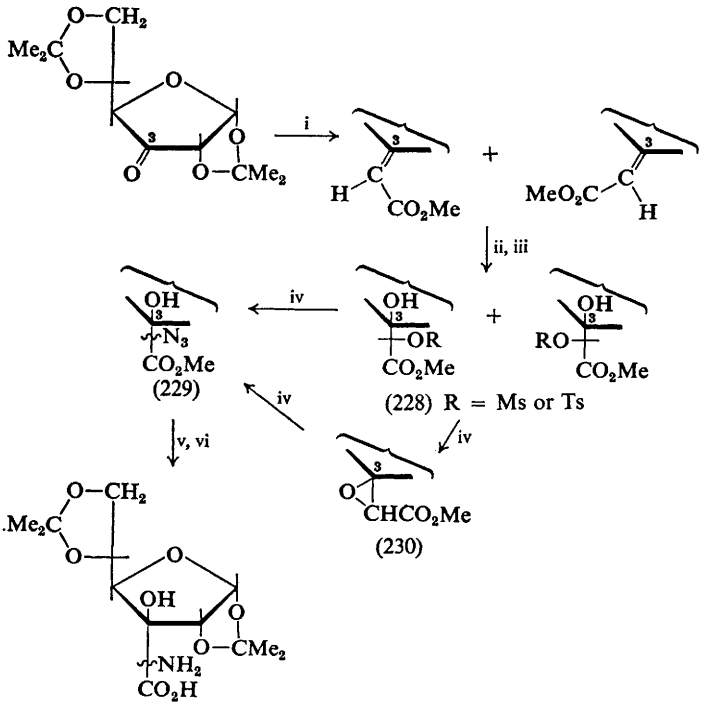
Reagents: i, MeMgI–Et_2O; ii, H_3O^+; iii, TsCl–py; iv, MeONa; v, $LiAlH_4$; vi, MeOH–H^+; vii, MeI; viii, MeOH–Me_2CO–H^+

Scheme 93

Grignard product (225) was demonstrated by formation of the di-*O*-isopropylidene methyl glycoside (226), albeit in low yield.[460]

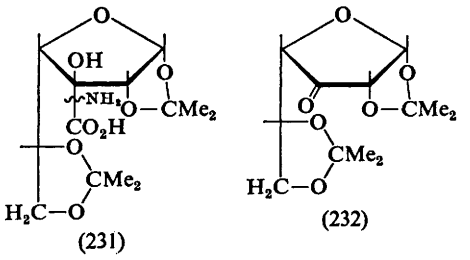
Rosenthal's group has been concerned with the synthesis of branched-chain sugars containing an amino-acid moiety attached by a carbon–carbon bond to C-3 of the sugar, which can be considered as structural analogues of the sugar moiety of the polyoxins. Compounds with both $R^1—C—OR^2$ and R—C—H branches have been obtained, and a synthesis of the first type is shown in Scheme 94.[461] Although a mixture of geometric isomers was obtained from the Wittig reaction, separation was unnecessary since subsequent treatment of either of the sulphonates (228) with azide ion produced a mixture of the azides (229). This reaction supposedly proceeds by two separate routes, *viz.* by simple displacement with inversion of configuration and by a double-inversion involving intermediate epoxides (230). A similar route was used to prepare a mixture of the D-*galacto*-analogues (231) from the hexosulose derivative (232).[462]

[459] P. K. Kindel and R. R. Watson, *Biochem. J.*, 1973, **133**, 227.
[460] J. S. Brimacombe, A. J. Rollins, and S. W. Thompson, *Carbohydrate Res.*, 1973, **31**, 108.
[461] A. Rosenthal, C. M. Richards, and K. Shudo, *Carbohydrate Res.*, 1973, **27**, 353.
[462] A. Rosenthal and C. M. Richards, *Carbohydrate Res.*, 1973, **29**, 413.



Reagents: i, $(MeO)_2P(O)CH_2CO_2Me$; ii, $KMnO_4$; iii, MsCl(TsCl)–py; iv, NaN_3–DMF; v, H_2–Pd; vi, NaOH

Scheme 94

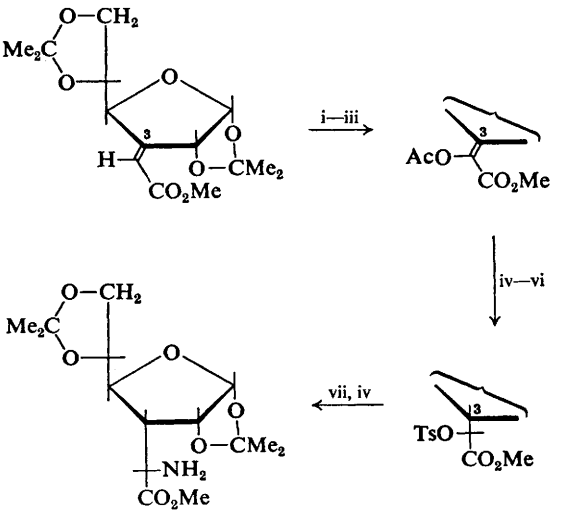


(231) (232)

Compounds with an R—C—H Branch

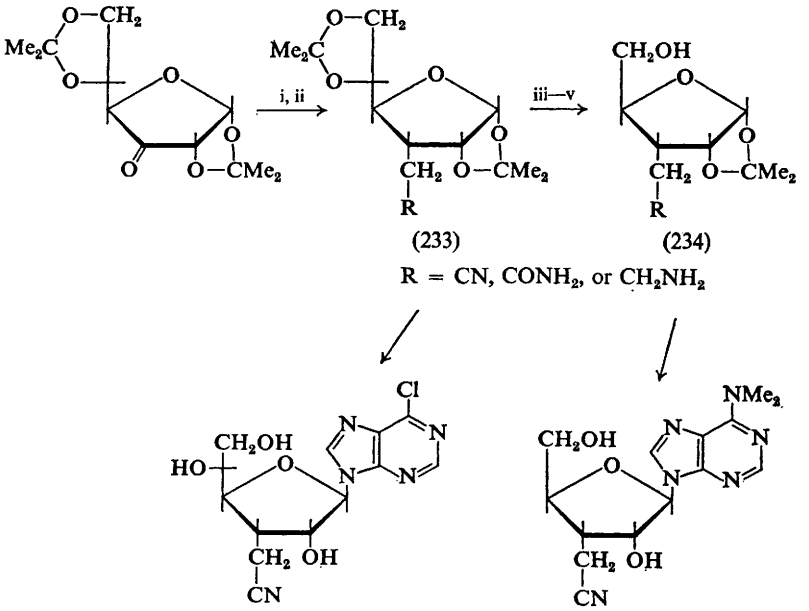
Continuing from the previous section, the synthesis of a 3-deoxy-analogue is shown in Scheme 95.[463] The azide displacement in this case proceeded normally, with inversion of configuration at the relevant carbon atom, and stereochemical assignments were confirmed by o.r.d. measurements. The

[463] A. Rosenthal and K. Shudo, *J. Org. Chem.*, 1972, **37**, 4391.



Reagents: i, $KMnO_4$; ii, Ac_2O–py; iii, $SOCl_2$; iv, H_2–Pd; v, NaOH; vi, TsCl–py; vii, NaN_3–DMF

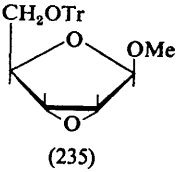
Scheme 95



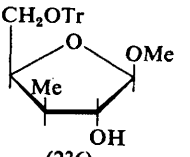
Reagents: i, $(EtO)_2P(O)CH_2CN$; ii, H_2–Pd; iii, H_3O^+; iv, $NaIO_4$; v, $NaBH_4$

Scheme 96

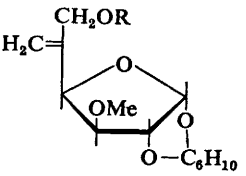
same workers have described a number of derivatives, including nucleosides, obtained from the branched-chain nitrile (233; R = CN);[464] conversion into the related pentose derivative (234; R = CN) yielded a similar set of compounds.[464a] Both series are summarized in Scheme 96, where examples of branched-chain derivatives of nucleosides are also shown. 9-(3-Deoxy-3-*C*-methyl-β-D-xylopyranosyl)adenine, a branched-chain analogue of cordycepin, has been synthesized.[465] A Grignard reaction on methyl 2,3-anhydro-5-*O*-trityl-β-D-ribofuranoside (235) furnished the required branched-chain precursor methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside (236).



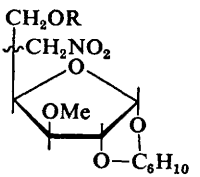
(235)



(236)



(237) R = Bn or Tr

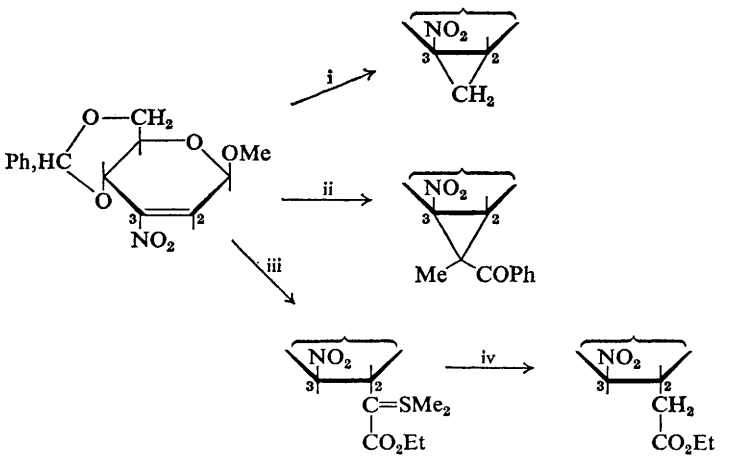


(238)

Addition of nitryl iodide to the branched-chain alkene (237) gave a tertiary iodide, which was hydrogenolysed with sodium borohydride to a separable mixture of the D-*gluco*- and L-*ido*-nitro derivatives (238).[466] Nitrocyclitols were obtained by cyclization of these derivatives after removal of the protecting groups. A novel series of branched-chain nitro-sugars, including derivatives containing cyclopropane rings, has resulted from the reactions of various ylides with an unsaturated nitro-sugar; the reactions are summarized in Scheme 97.[371]

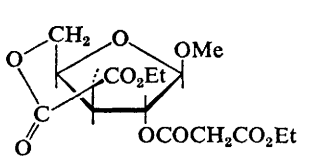
Lithium dimethyl cuprate has proved to be an excellent reagent for cleaving epoxides, with the formation of *C*-methyl derivatives.[177] Hanessian's group has examined the reaction of diethyl sodiomalonate with sugar epoxides and sulphonates; as well as nucleophilic substitution reactions, ester-exchange also occurred.[467] Reaction of diethyl sodiomalonate in boiling benzene with methyl 2,3-anhydro-β-D-ribofuranoside gave the lactone derivative (239), while reaction with methyl 3-*S*-alkyl-4,6-*O*-benzylidene-3-thio-2-*O*-toluene-*p*-sulphonyl-α-D-altropyranosides

[464] A. Rosenthal and D. A. Baker, *J. Org. Chem.*, 1973, **38**, 193.
[464a] A. Rosenthal and D. A. Baker, *J. Org. Chem.*, 1973, **38**, 198.
[465] S. R. Jenkins and E. Walton, *Carbohydrate Res.*, 1973, **26**, 71.
[466] J. Yoshimura, T. Iida, H. Wakia, and M. Funabashi, *Bull. Chem. Soc. Japan*, 1973, **46**, 3207.
[467] S. Hanessian, P. Dextraze, and R. Masse, *Carbohydrate Res.*, 1973, **26**, 264.

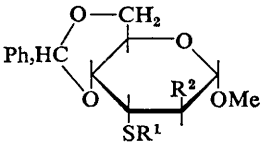


Reagents: i, Me_2SO; ii, $Me_2S{=}CMeCOPh$; iii, $Me_2S{=}CHCO_2Et$; iv, H_2–cat.

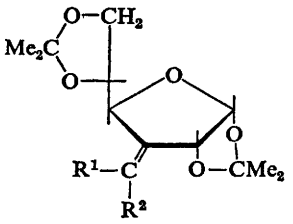
Scheme 97



(239)



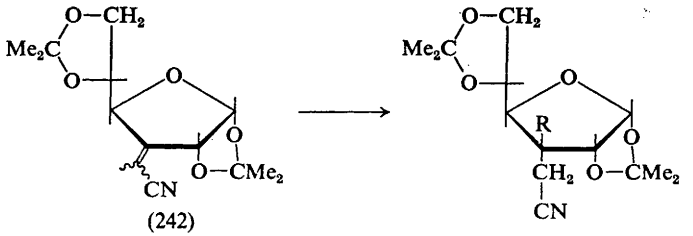
(240) R^1 = Et, Bn, Ph; R^2 = $CH(CO_2Et)_2$



(241) R^1,R^2 = H, X or X, H(X = Cl, Br, or I)

afforded 2-*C*-(diethyl malonyl) derivatives (240) *via* an intermediate episulphonium ion.

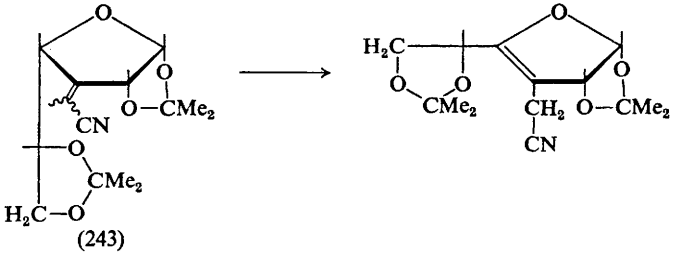
The halogenated, branched-chain alkenes (241) have been derived from the corresponding hexofuranos-3-ulose using Wittig reagents; mixtures of *cis*- and *trans*-isomers were separated, and configurations were assigned on the basis of n.m.r. spectroscopic evidence.[468] The reactions of the Wittig products (242) and (243) with nucleophiles have been compared; additions were preferred with (242), whereas isomerization occurred with (243) (see Schemes 98 and 99).[469] It was suggested that formation of an



(242)

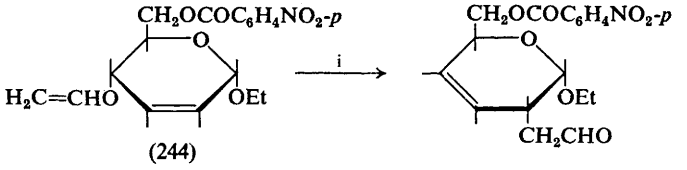
R = CN, OMe, SPh, or NHBn

Scheme 98



(243)

Scheme 99



(244)

Reagent: i, heat–$PhNO_2$

Scheme 100

[468] J. M. J. Tronchet and D. Schwarzenbach, *Carbohydrate Res.*, 1973, **30**, 395.
[469] J. M. J. Tronchet and J.-M. Bourgeois, *Carbohydrate Res.*, 1973, **29**, 373.

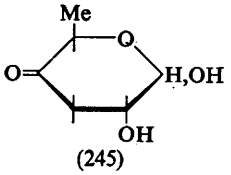
sp^3-hybridized carbon atom at position 3 of (243) is impeded by the *endo*-substituent at C-4. A number of syntheses of the branched-chain, conjugated diene (202) have been reported [434] (see Chapter 14).

A novel synthesis of unsaturated, branched-chain sugars has utilized a Claisen rearrangement of 2,3-dideoxy-4-*O*-vinyl-hex-2-enopyranoside derivatives [*e.g.* (244)] to give isomeric 2,3,4-trideoxy-2-*C*-(formylmethyl)-hex-3-enopyranosides (Scheme 100); the rearrangement was also performed on the 6-benzoylated, C-4 epimer of (244) to give ethyl 6-*O*-benzoyl-2,3,4-trideoxy-2-*C*-(formylmethyl)-α-D-*threo*-hex-3-enopyranoside.[194]

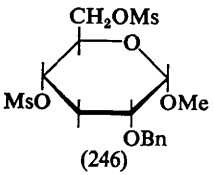
16
Aldehydo-sugars, Aldosuloses, and Diuloses

The mechanism of the condensation of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose with ethyl nitroacetate has been investigated.[470]

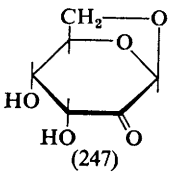
An aldosulose, a 2,3,6-trideoxy-L-hexos-4-ulose, has been isolated from the products of hydrolysis of the antibiotic B-58941.[471] 3,6-Dideoxy-D-*erythro*-hexos-4-ulose (245) has been synthesized from the 3-deoxy-D-*ribo*-hexoside (246) by standard methods, and has been shown to be one of the intermediates on the biosynthetic pathway for the conversion of D-glucose into 3,6-dideoxyhexoses in bacteria.[472] The 1,6-anhydro-D-aldohexosuloses (247) and (248) were prepared by oxidation of the appropriate isopropylidene derivatives, and both compounds were shown to exist



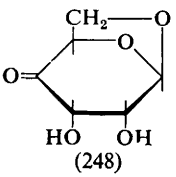
(245)



(246)



(247)



(248)

in solution as dimeric hemiacetals (*cf.* Vol. 5, p. 107).[473] Somewhat unexpectedly, catalytic oxidation of sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose) (44) over platinum gave the 4-ulose (46) in which C-5 had undergone epimerization[188] (see Scheme 21). It was suggested that the 5-ulose (45) is the initial product of oxidation, in keeping with the

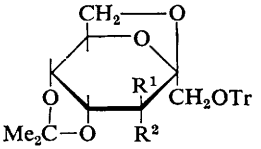
[470] V. I. Kornilov, B. B. Paidak, and Yu. A. Zhdanov, *Zhur. obshchei Khim.*, 1973, **43**, 189.

[471] T. Suzuki, N. Sugita, and M. Asai, *Chem. Letters*, 1973, 789 (*Chem. Abs.*, 1973, **79**, 115 838c).

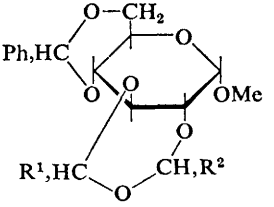
[472] C. L. Stevens, K. W. Schultze, D. J. Smith, P. M. Pillai, P. Rubenstein, and J. L. Strominger, *J. Amer. Chem. Soc.*, 1973, **95**, 5767.

[473] K. Heyns and P. Köll, *Chem. Ber.*, 1973, **106**, 611.

preferential catalytic oxidation of axial hydroxy-groups, but that it then isomerizes to the 4-ulose. Reduction of the 4-ulose (46) with sodium borohydride afforded 2,7-anhydro-β-D-*talo*-heptulopyranose (47). Oxidation of the sedoheptulosan derivative (249) with ruthenium tetroxide gave the corresponding 3-ulose, borohydride reduction of which gave the configurational isomer (250).[474]



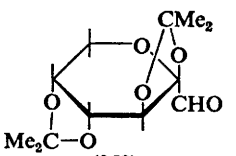
(249) R^1 = OH, R^2 = H
(250) R^1 = H, R^2 = OH



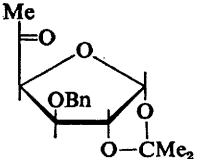
(251) R^1 = R^2 = H
(252) R^1 = Ph, R^2 = H

The 2- and 5-ulose derivatives of methyl α- and β-D-glucofuranosidurono-6,3-lactones have been obtained by a variety of methods, both from the parent compounds and from partially protected derivatives thereof.[475] A 5-ulose derivative was also prepared by oxidation of 1,2-*O*-cyclohexylidene-(isopropylidene)-α-D-glucofuranurono-6,3-lactone. The oxidized products were isolated as the hydrated forms and their reducing properties were examined.

Oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with phosphorus pentoxide–DMSO yielded the corresponding 3-ulose, together with the acetalated products (251) and (252), each containing a seven-membered ring spanning the 2,3-diol grouping.[476] Formaldehyde necessary for the reaction was assumed to have been formed from DMSO, whereas benzaldehyde presumably arose from partial hydrolysis of the starting material. Aldehydic derivatives [*e.g.* (253)] and ketonic derivatives [*e.g.* (254)] have been obtained in good yield by oxidation of the appropriate alcohols with a chromium trioxide–bipyridyl complex.[477] Such homologous aldehydic derivatives as (174, R = CHO) have been obtained by way of the reaction of primary iodides with 2-lithio-1,3-dithian.[394]



(253)



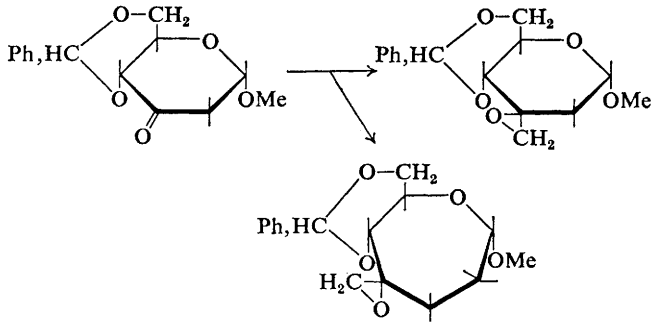
(254)

[474] K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, 1973, **106**, 1668.
[475] K. Dax and H. Weidman, *Carbohydrate Res.*, 1972, **25**, 363.
[476] Y. Kondo and F. Takao, *Canad. J. Chem.*, 1973, **51**, 1476.
[477] R. E. Arrick, D. C. Baker, and D. Horton, *Carbohydrate Res.*, 1973, **26**, 441.

The formation of aldosuloses and diuloses from γ-irradiation of oxygen-free solutions of D-glucose and D-fructose [35] is noted in Chapter 2.

In continuing their work on sugar oximes, Lemieux and his co-workers have examined methods for converting the oximo-group into a carbonyl group.[387] The preferred method involved the use of a slight excess of acetaldehyde in acetonitrile in the presence of an equivalent of hydrogen chloride; deoximation of (169) by this procedure gave the crystalline ketone (19) in high yield. The stereochemical results of the reduction of isopropyl 3,4,6-tri-*O*-acetyl-α-D-*arabino*-hexopyranosid-2-ulose (19) and the D-*lyxo*-isomer (20) are tabulated in Chapter 2.[89]

Full details have appeared of the work by Overend's group on the action of diazomethane on glycosuloses (*cf.* Vol. 1, p. 140); the reaction of diazomethane with methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose, to give both pyranosidic and septanosidic products, was also described (Scheme 101).[478] Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-

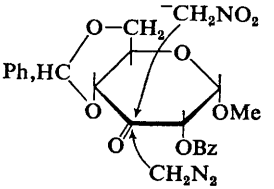


Scheme 101

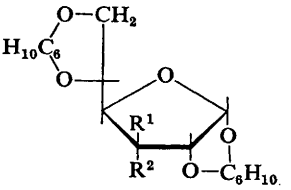
ribo-hexopyranosid-3-ulose (255) underwent equatorial attack at the carbonyl group by nitromethane, whereas axial attack was favoured by diazomethane; the stereochemistries of the products were ascertained by conversion into known compounds.[479] Condensation of 1,2:5,6-di-*O*-cyclohexylidene-α-D-*ribo*-hexofuranos-3-ulose (256) with ethyl bromoacetate in a Reformatski reaction gave, after saponification, 3-*C*-carboxymethyl-1,2:5,6-di-*O*-cyclohexylidene-α-D-allofuranose (257).[480]

Tronchet's group has noted that epimerization sometimes accompanies Wittig reactions on aldosulose derivatives; whether or not epimerization occurs seems to depend on the aldosulose derivative and phosphorane employed.[481] For example, the D-*ribo*-3-ulose (258) underwent partial

[478] B. Flaherty, S. Nahar, W. G. Overend, and N. R. Williams, *J.C.S. Perkin I*, 1973, 632.
[479] J. Yoshimura, K. Sato, K. Kobayashi, and C. Shin, *Bull. Chem. Soc. Japan*, 1973, **46**, 1515.
[480] Yu. A. Zhdanov, Yu. K. Alexeev, and Kh. A. Kurdanov, *Zhur. obshchei Khim.*, 1973, **43**, 186.
[481] J. M. J. Tronchet, J. M. Bourgeois, and D. Schwarzenbach, *Carbohydrate Res.*, 1973, **28**, 129.

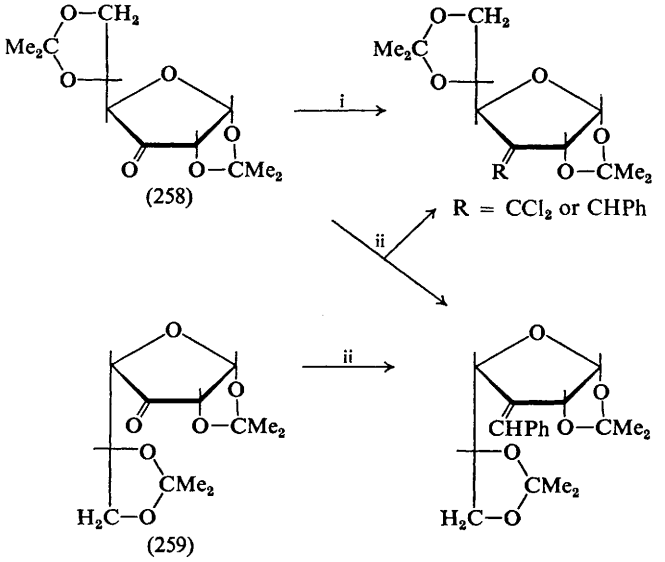


(255)



(256) R^1, R^2 = O
(257) R^1 = $-CH_2CO_2H$, R^2 = OH

epimerization at C-4 to give four products on reaction with benzylidene-triphenylphosphorane, whereas epimerization was not observed with the D-*xylo*-analogue (259) (see Scheme 102). However, the D-*ribo*-compound (258) suffered no epimerization at C-4 when a different phosphorane was used.



(258)

R = CCl_2 or CHPh

(259)

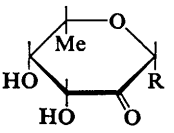
Reagents: i, $Ph_3P{=}CCl_2$; ii, $Ph_3P{=}CHPh$

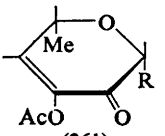
Scheme 102

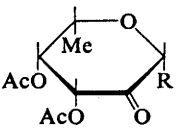
Methyl 2,3-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-*lyxo*-hexofuranosid-5-ulose has been prepared, and it reacted with methanol in the presence of triethylamine to give a dimethyl acetal (54) and a derivative of an aldodiulose (55) (see Scheme 25).[195]

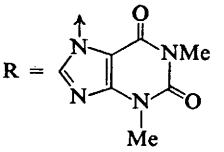
Acetylation of the keto-nucleoside (260) resulted mainly in β-elimination of an acetoxy-group to furnish the enol acetate (261), although some diacetate (262) was formed.[482]

[482] K. Antonakis and M.-J. Arvor-Egron, *Carbohydrate Res.*, 1973, **27**, 468.


(260)

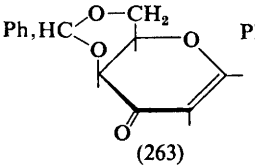

(261)

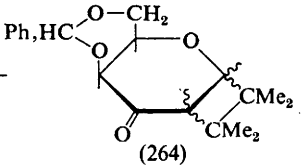

(262)

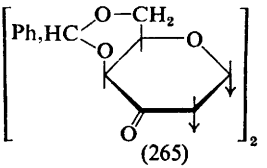


Photoaddition of 2,3-dimethylbut-2-ene to the enone (263) yielded three isomeric cyclobutanes (264) (*eq.*, *eq.*; *eq.*, *ax.*; and *ax.*, *eq.*) and a dimer (265).[483] Similar addition to the C-4 epimer of (263) gave the corresponding cyclobutanes, but dimeric products did not appear to be formed. A number of other enones are referred to in Chapter 14.

The first syntheses of acetylated glycopyranosyl-2-ulose chlorides are reported in Chapter 7.


(263)


(264)


(265)

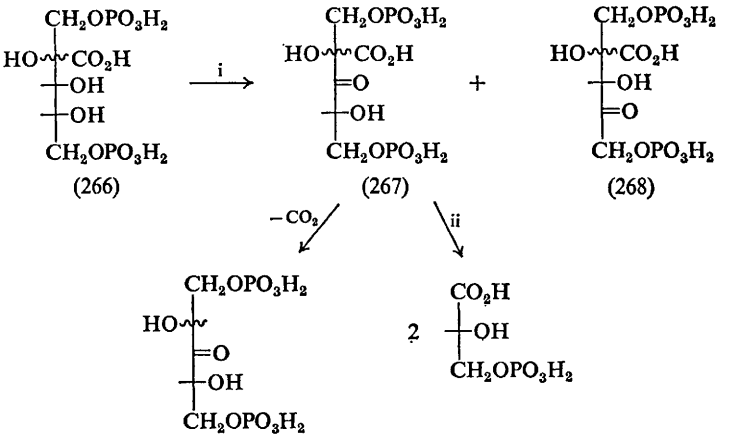
[483] P. M. Collins and B. R. Whitton, *J.C.S. Perkin I*, 1973, 1470.

17
Sugar Acids and Lactones

Aldonic Acids

Dicyclohexylamine can be used with non-nitrogen-containing aldonolactones to give dicyclohexylammonium aldonates, which are usually satisfactory crystalline derivatives for the isolation and characterization of aldonic acids. 2-Acetamido-2-deoxy-D-mannono-1,4-lactone also reacted with the amine, but, in this case, epimerization occurred and the dicyclohexylammonium salt of 2-acetamido-2-deoxy-D-gluconic acid was isolated.[484]

A detailed analysis of the hydrolysis of D-glucono-1,5-lactone has been reported; general acid and general base catalysis were observed.[485] Branched-chain 2-deoxyaldonic acids have been prepared by way of the Reformatski reaction,[457] and the branched-chain acid derivative (266) on catalytic oxidation gave both possible carbonyl products (267) and (268) (Scheme 103).[486] The former is the proposed intermediate in the enzymic



Reagents: i, Pt–C–O_2–Mg^{2+}; ii, ribulose diphosphate carboxylase

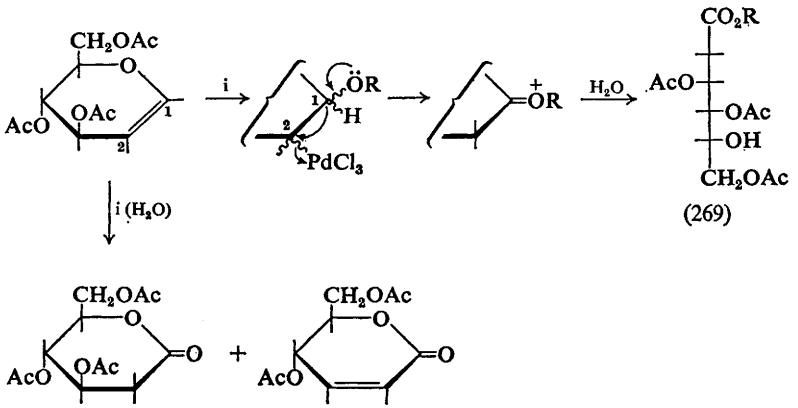
Scheme 103

[484] E. Zissis, H. W. Diehl, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1973, **26**, 323.
[485] Y. Pocker and E. Green, *J. Amer. Chem. Soc.*, 1973, **95**, 113.
[486] M. L. Siegel and M. D. Lane, *J. Biol. Chem.*, 1973, **248**, 5486.

carboxylation of D-ribulose 1,5-diphosphate, and it is significant that chemically synthesized (267) was cleaved by ribulose diphosphate carboxylase to give two molecules of D-glyceric acid 3-phosphate.

The synthesis of 2-amino-5-*O*-carbamoyl-2-deoxy-L-xylonic acid, a derivative of the acyclic component of polyoxin A, has been prepared by a lengthy but orthodox route from an L-sorbose derivative.[487]

γ-Irradiation of aqueous solutions of D-glucose afforded 2-deoxy-D-*arabino*-hexono-1,4-lactone, and the methyl ester of the same acid was also isolated.[488] Oxidation of 3,4,6-tri-*O*-acetyl-D-glucal in alcohols in the presence of $PdCl_4^{2-}$ and a copper salt gave the ester (269).[489] A hydride shift occurred, since H-1 of the glycal was shown by labelling experiments to migrate to C-2. In aqueous media, an unsaturated lactone was produced (Scheme 104).



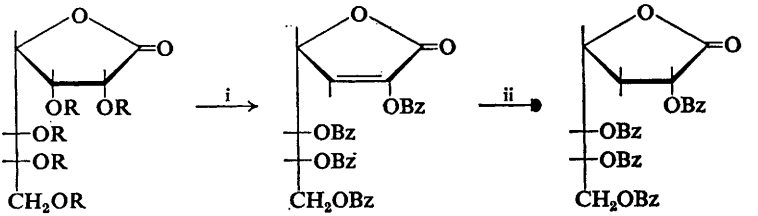
Reagent: i, $PdCl_4^{2-}$–$Cu(NO_3)_2$–ROH

Scheme 104

Benzoylation of D-*glycero*-D-*gulo*-heptono-1,4-lactone gave the expected pentabenzoate over short reaction periods, but elimination of the 3-benzoyloxy-group occurred on prolonged reaction with an excess of benzoyl chloride. Reduction of the unsaturated lactone gave the 3-deoxy-product (270) (Scheme 105).[490]

Continued studies with 2-acetamido-2-deoxy-D-mannose have shown that oxidation to the aldonic acid with bromine water gives the expected product, but when the 1,4-lactone was isolated and treated with a secondary amine, some D-gluconic acid salt (see also ref. 484) and the enamide (271) were formed.[491] A related enamide is described in Chapter 14.

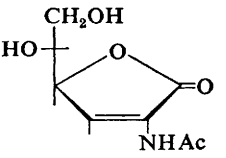
[487] H. Kuzuhara and S. Emoto, *Tetrahedron Letters*, 1973, 5051.
[488] S. Kawakishi and M. Namiki, *Carbohydrate Res.*, 1973, **26**, 252.
[489] M. Gouedard, F. Gaudemer, and A. Gaudemer, *Bull. Soc. chim. France*, 1973, 577.
[490] M. I. Litter and R. M. de Lederkremer, *Carbohydrate Res.*, 1973, **26**, 431.
[491] E. Zissis, H. W. Diehl, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1973, **28**, 327.



R = H → R = Bz (270)

Reagents: i, BzCl–py; ii, H_2–Pd–C

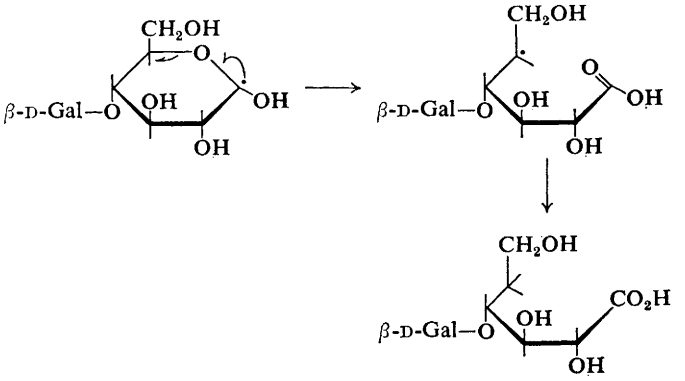
Scheme 105



(271)

In the disaccharide series, γ-irradiation of α-lactose has been shown to give 5-deoxylactobionic acid (Scheme 106).[492]

The oxidation of D-glucose to D-gluconic acid is mentioned in Chapter 22.



Scheme 106

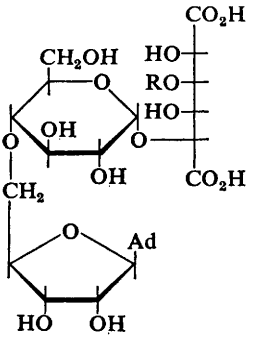
Aldaric Acids

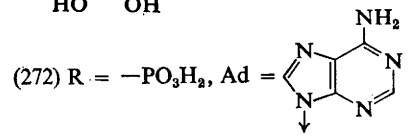
D-Galactaric acid has been identified in aqueous extracts obtained from the succulent *Ferocactus acanthodes*.[493] Because of its insolubility, it is unlikely that the acid exists in the free form in the plant, but the isolation procedure is consistent with the acid existing naturally as a salt or as a lactone.

[492] M. Dizdaroglu, C. von Sonntag, D. Schulte-Frohlinde, and W. V. Dahlhoff, *Annalen*, 1973, 1592.

[493] R. Kringstad and A. Nordal, *Acta Chem. Scand.*, 1973, **27**, 1432.

The point of attachment of the allaric-acid component of a bacterial exotoxin (272) has been established by methylation and periodate-oxidation studies.[494]



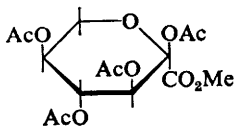


(272) R = $-PO_3H_2$, Ad =

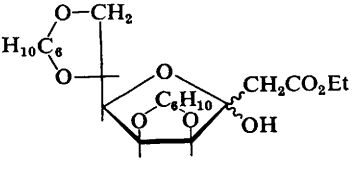
Ulosonic Acids

3-Deoxy-D-*threo*-hex-2-ulosonic acid and 3-deoxy-D-*arabino*-hept-2-ulosonic acid have been synthesized by oxidation of the appropriate 3-deoxyaldonic acids.[495] Acid treatment of these ulosonic acids gave enols of the corresponding 1,4-lactones and then furan derivatives. 3-Deoxy-D-*erythro*-hex-2-ulosonic acid has been prepared with uniform ^{14}C-labelling and also with labelling at C-1 specifically.[496] Also in the the hex-2-ulosonic acid series, acid degradation of the α-L-*xylo*-compound (273) has been examined in detail;[497] compound (274) has been prepared in the oct-3-ulosonic acid series.[498]

On heating in aqueous solution at 100 °C and pH 7, 4-*O*-methyl-D-glucuronic acid isomerized to 3-*O*-methyl-D-*lyxo*-hex-5-ulosonic acid



(273)



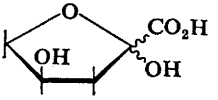
(274)

[494] L. Kalvoda, M. Prystaš, and F. Šorm, *Tetrahedron Letters*, 1973, 1873.
[495] D. Charon and L. Szabo, *J.C.S. Perkin I*, 1973, 1175.
[496] J. Pouyssegur, *J. Labelled Compounds*, 1973, **9**, 1.
[497] K. Goshima, N. Maezono, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1972, **45**, 3692.
[498] Yu. A. Zhdanov, Yu. E. Alekseev, and Kh. A. Kurdanov, *J. Gen. Chem.* (*U.S.S.R.*), 1972, **42**, 2767.

(47%), 3-*O*-methyl-L-*ribo*-hex-5-ulosonic acid (12%), 4-*O*-methyl-D-mannuronic acid (4%), and 3-*O*-methyl-L-*ribo*-hex-4-ulosonic acid (1%).[43]

The calcium salt of D-*threo*-hex-2,5-diulosonic acid, prepared by microbial oxidation of D-glucose, gave *N*-substituted 5-oxidopyridazinium derivatives on treatment with methyl- and aryl-hydrazines.[499]

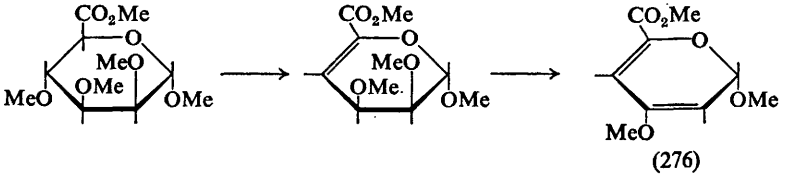
3-Deoxy-L-*glycero*-pent-2-ulosonic acid (275) has been identified as a component of the capsular polysaccharide of a *Klebsiella* strain.[500]



(275)

Uronic Acids

Further work on elimination reactions applied to hexuronic acid esters has appeared. Methyl esters of fully methylated D-glycopyranosiduronic acids, prepared from the glycosides by oxidation with potassium ferrate or with sodium hypoiodite followed by methylation, were treated with sodium methoxide in methanol and gave 4,5-unsaturated products, irrespective of the configuration of the 4-methoxy-group; in the case of the *manno*-product, further *trans*-elimination occurred to give the pyran (276) (Scheme 107).[501] The probable mechanism (*E*1cB) of elimination was discussed. A



(276)

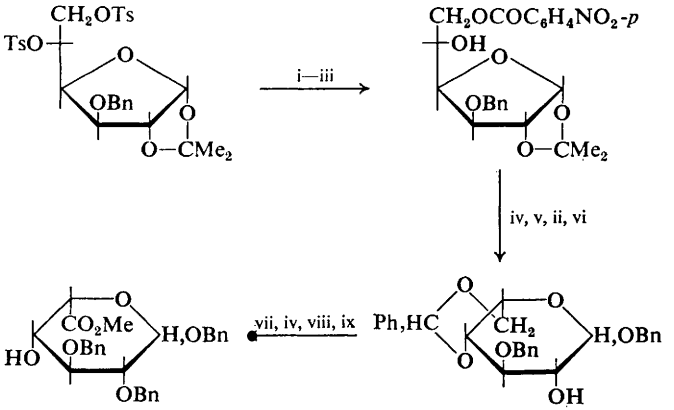
Scheme 107

decarboxylation-elimination reaction of a uronic acid is reported in Chapter 14.

The synthesis of the anomeric methyl (benzyl 2,3-di-*O*-benzyl-L-idopyranosid)uronates has been carried out as shown in Scheme 108.[502]

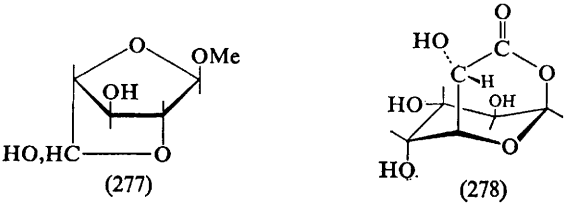
Controlled periodate oxidation of methyl β-D-galactofuranoside gave the expected aldehydic product, which exists in the hemiacetal form (277). A cyanohydrin synthesis based on (277) provided a route to D-galacturonic and L-altruronic acids, and these were separated by chromatography; the use of malonic acid (Knoevenagel–Doebner synthesis) gave D-*glycero*-L-

[499] K. Imada, *J.C.S. Chem. Comm.*, 1973, 796.
[500] B. Lindberg, K. Samuelsson, and W. Nimmich, *Carbohydrate Res.*, 1973, **30**, 63.
[501] J. N. BeMiller and G. V. Kumari, *Carbohydrate Res.*, 1972, **25**, 419.
[502] J. Kiss and P. C. Wyss, *Carbohydrate Res.*, 1973, **27**, 282.



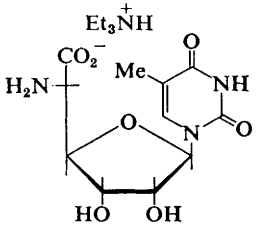
Reagents: i, $KOAc-Ac_2O$; ii, $MeONa-MeOH$; iii, $p-NO_2C_6H_4COCl-py$; iv, $AcOH-H_2O$; v, $BnOH-H^+$; vi, PhCHO; vii, BnCl–KOH; viii, O_2–Pt; ix, CH_2N_2

Scheme 108



altro-hepturonic acid (278) following oxidation, with iodic acid and osmium tetroxide, of the initially formed, unsaturated heptonic acid.[503]

The total synthesis of polyoxin J has been described;[504] the aldonic-acid portion used has been reported in the section on 'Aldonic Acids', whereas the hexuronic-acid component used was the salt (279) (see Chapter 20 for details). Related 5-aminohexuronic acids have been mentioned elsewhere.[404]



(279)

[503] O. Kjolberg and T. B. Sverreson, *Acta Chem. Scand.*, 1972, **26**, 3245.
[504] H. Kuzuhara, H. Ohrui, and S. Emoto, *Tetrahedron Letters*, 1973, 5055.

Photolytic cleavage of a D-glucuronide linkage in a saponin has been reported, the carbohydrate product being a 5-deoxyuronic acid [505] (*cf.* Scheme 106).

A novel synthesis of a penturonic ester has been described. Treatment of 1,2-*O*-isopropylidene-α-D-glucofuranose in boiling methanol with silver carbonate on Celite gave a good yield of methyl (1,2-*O*-isopropylidene-α-D-xylofuranos)uronate.[506]

3-*O*-(β-D-Glucopyranosiduronic acid)-D-glucono-1,4-lactone has been prepared for use as an inhibitor of β-glucuronidase.[507]

Russian workers have reported on t-butyl and t-amyl peresters of D-galacturonic acid,[508] and have prepared the acetylated glycosyl chloride [509] and a glycosylamine [509a] of D-mannuronic acid.

Various glycopyranuronates are reported in Chapter 3 and various glycuronosyl nucleosides are described in Chapter 21.

Ascorbic Acids

An improved procedure for preparing L-ascorbic acid 2-sulphate has been published,[510] and a series of optically active tetronic acids, which are somewhat related to ascorbic acid, has been described.[511]

Diphenyl selenoxide has been used to oxidize L-ascorbic acid to the dehydro-compound, which was isolated as its 2,4-dinitrophenylosazone.[512] The photo-oxidation of L-ascorbic acid in water at various pH values has been studied (using e.s.r. spectroscopy), both in the presence and absence of oxygen, nitrous oxide, and hydrogen peroxide; two distinct radicals were detected as intermediates.[513] The results are consistent with a primary photochemical step involving ejection of an electron from the monoanion of L-ascorbic acid to form the ascorbate, whereas the second radical appears to be formed by addition of an hydrated electron to the monoanion.

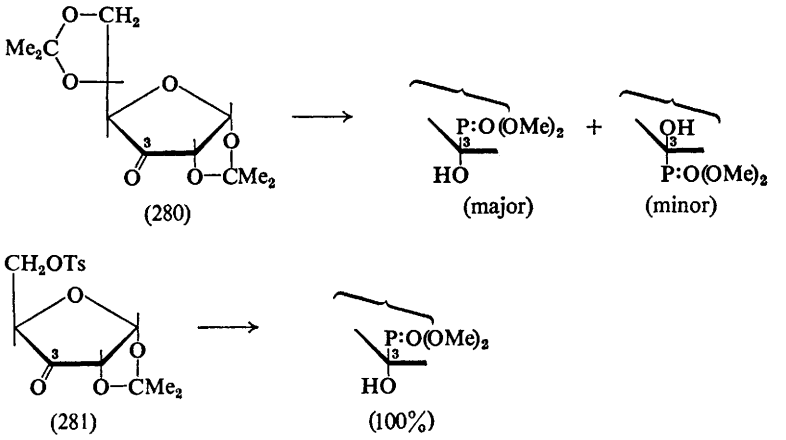
[505] I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, 1973, 3997.
[506] S. Morgenlie, *Acta Chem. Scand.*, 1973, **27**, 2217.
[507] I. Matsunaga and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1218.
[508] G. S. Bylina and L. P. Uvarova, *Zhur. org. Khim.*, 1972, **8**, 2520.
[509] L. G. Revelskaya, A. N. Anikeeva, and S. N. Danilov, *Zhur. obshchei Khim.*, 1972, **42**, 2304.
[509a] L. G. Revelskaya, A. N. Anikeeva, and S. N. Danilov, *Zhur. obshchei Khim.*, 1973, **43**, 1624.
[510] S. F. Quadri, P. A. Seib, and C. W. Deyoe, *Carbohydrate Res.*, 1973, **29**, 259.
[511] J. L. Bloomer and F. E. Kappler, *Tetrahedron Letters*, 1973, 163.
[512] I. Perina, N. Bregant, and K. Balenovic, *Bull. Sci. Conseil Acad. Sci. Arts R.S.F., Yougoslavie, Section A*, 1973, **18**, 4 (*Chem. Abs.*, 1973, **78**, 160 007w).
[513] R. D. McAlpine, M. Cocivera, and H. Chen, *Canad. J. Chem.*, 1973, **51**, 1682.

18
Inorganic Derivatives

Carbon-bonded Compounds

Several reports have appeared during 1973 on nucleophilic displacements effected with dialkyl phosphites. An allylic displacement, occurring when 3,4,6-tri-*O*-acetyl-D-glucal was treated with dimethyl phosphite, is mentioned in Chapter 14.

Hall and his colleagues have extended their studies on the reaction of aldosuloses with dimethyl phosphite. Measurements of ^{31}P–^{1}H couplings in the products showed that attack on the carbonyl group of the aldos-3-uloses (280) and (281) generally occurs from the less-hindered side of the bicyclic system, as illustrated in Scheme 109.[514] Paulsen's group has reported

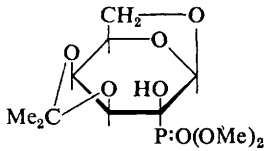


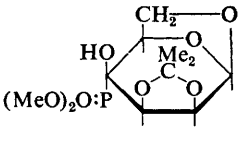
Scheme 109

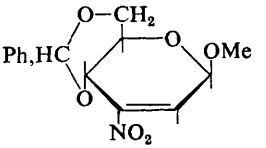
syntheses of the phosphonates (282) and (283) (and others), and has proposed a relationship between $J_{P,H}$ for the system PCOH and the dihedral angle between P–C and O–H bonds.[515] Additions of dimethyl phosphite

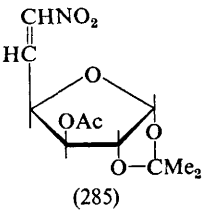
[514] L. Evelyn, L. D. Hall, L. Lynn, P. R. Steiner, and D. H. Stokes, *Carbohydrate Res.*, 1973, **27**, 21.

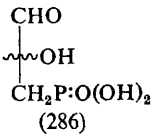
[515] H. Paulsen and W. Greve, *Chem. Ber.*, 1973, **106**, 2124.

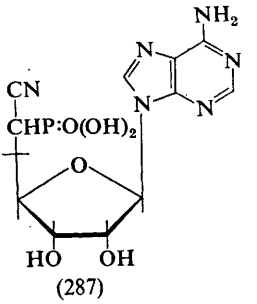

(282)


(283)


(284)

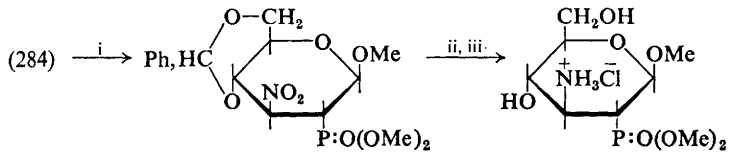

(285)


(286)


(287)

to the nitro-olefins (284) and (285) have been used to obtain amino-sugar phosphonates (see Scheme 110).[516]

A synthesis of DL-glyceraldehyde-3-phosphonic acid (286) has been achieved by standard procedures involving, in the key step, nucleophilic displacement of a sulphonyloxy-group with triethyl phosphite.[517] The



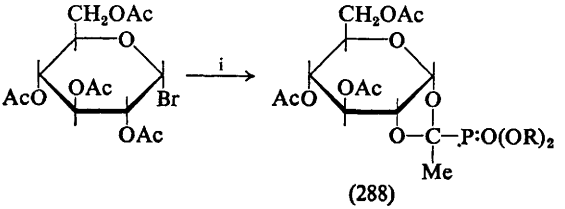
Reagents: i, $HP{:}O(OMe)_2$–Et_3N; ii, H^+; iii, H_2–Pt

Scheme 110

1,3-diphosphonic acid of glycerol[518] and a nucleotide analogue (287)[519] have been similarly prepared. As structural analogues of naturally occurring phosphates, the phosphonates are of interest as potential antimetabolites.

Related compounds, containing the phosphorus atom bonded directly to the carbohydrate skeleton, have also been described by Paulsen and Thiem. Whereas 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide underwent elimination to form the hex-1-enopyranose on treatment with alkyl phosphites, the acetal phosphonate (288) was obtained with the bromomercury compound shown in Scheme 111.[520] By contrast, reaction of

[516] H. Paulsen and W. Greve, *Chem. Ber.*, 1973, **106**, 2114.
[517] E. Baer and R. Robinson, *Canad. J. Chem.*, 1973, **51**, 104.
[518] R. Robinson and E. Baer, *Canad. J. Biochem.*, 1973, **51**, 1203.
[519] A. Hampton, T. Sasaki, and B. Paul, *J. Amer. Chem. Soc.*, 1973, **95**, 4404.
[520] H. Paulsen and J. Thiem, *Chem. Ber.*, 1973, **106**, 115.

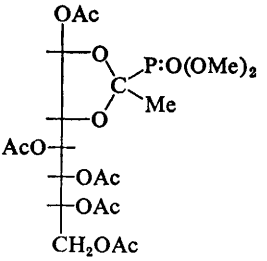


(288)

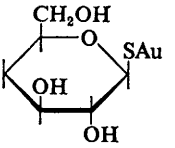
Reagents: i, $BrHgP{:}O(OR)_2$

Scheme 111

2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with silver dimethyl phosphite afforded the β-D-glucosyl phosphite ester, which then furnished the glycosyl phosphate on oxidation. This work was extended to other glycosyl (D-galactosyl, D-xylosyl, D-arabinosyl) halides, and the acetal phosphonate (289) was obtained in the acyclic series.[521]



(289)



(290)

Novel inorganic derivatives of carbohydrates, including the gold compound (290) (not unambiguously characterized),[522] and the thioarsenic (31, X = S) and selenoarsenic derivatives (31, X = Se) have been reported.[142]

Oxygen-bonded Compounds

Pulsed n.m.r. spectroscopic methods have been used to investigate the binding of sodium ions with the hydroxy-groups of free sugars and two inositols.[523] Changes in the ^{23}Na spin–lattice relaxation times indicated that weak interactions occur and that sodium and calcium ions compete for the same sites when present together in solution.

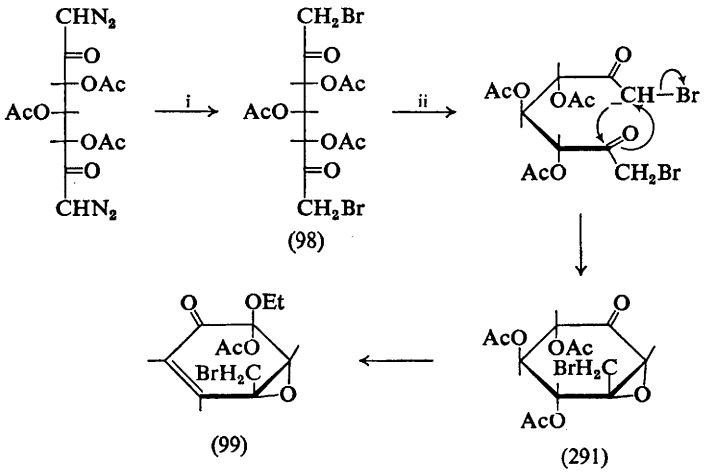
Complexation of Group II metal ions with nucleosides in DMSO solution has been studied by n.m.r. spectroscopy; differences between the sites of binding of Group IIa and IIb metal ions were noted.[524]

[521] H. Paulsen and J. Thiem, *Chem. Ber.*, 1973, **106**, 132.
[522] L. Vegh and E. Hardegger, *Helv. Chim. Acta*, 1973, **56**, 2079.
[523] J. Andrasko and S. Forsén, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 233.
[524] S. Shimokawa, H. Fukui, J. Sohma, and K. Hotta, *J. Amer. Chem. Soc.*, 1973, **95**, 1777.

19
Cyclitols

O-Methyl-*scyllo*-inositol has been isolated from the seeds of mung bean (*Phaseolus vidissimus*).[525] The cyclitol components of cannabis from various sources have been determined (see Chapter 2).[7, 8] Theoretical calculations on the conformations of cyclitols in solution have been made by MO–LCAO methods, and the order of stability for the cyclitols studied was determined as *myo* > *scyllo* > *neo* > *allo* > *muco* > *epi* > *cis*.[526] The crystal and molecular structures of a hydrated complex of *myo*-inositol and magnesium chloride showed that the six-membered ring is somewhat distorted from the shape assumed in the free cyclitol[527] (see also Chapter 24).

In an attempt to prepare a dicarbonyl sugar (*xylo*-hepto-2,6-diulose), the compound (98) was treated with sodium acetate in ethanol in an effort to



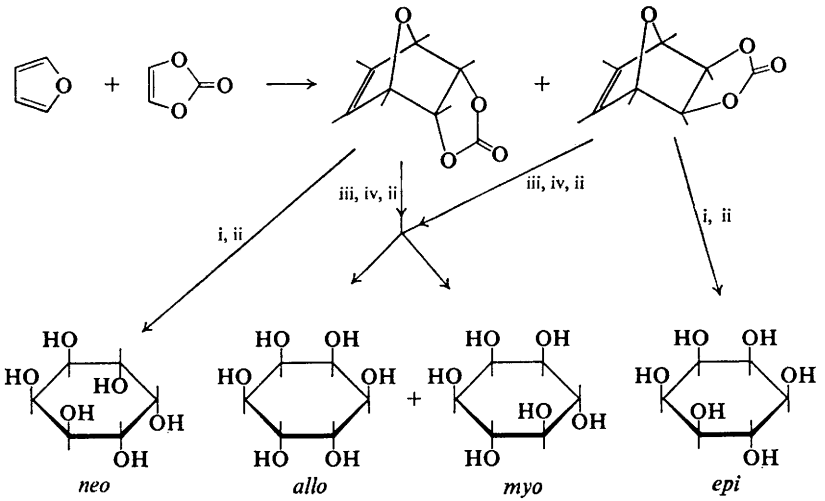
Reagents: i, HBr; ii, NaOAc–EtOH

Scheme 112

[525] Y. Ueno, A. Hasegawa, and T. Tsuchiya, *Carbohydrate Res.*, 1973, **29**, 520.
[526] K. S. Vijayalakshmi and V. S. R. Rao, *Proc. Indian Acad. Sci. Section A*, 1973, **77**, 83.
[527] G. Blank, *Acta Cryst.*, 1973, **B29**, 1677.

displace the bromo-substituents.[299] The structure of the cyclic product (99) resulting unexpectedly from this treatment was confirmed by spectroscopic and *X*-ray methods, and it was assumed that the cyclic ketone (291) is formed initially (Scheme 112).

Total, stereoselective syntheses of *myo*-, *allo*-, *neo*-, and *epi*-inositols have been accomplished by the reactions shown in Scheme 113; the intermediate 1,4-anhydroinositols were also isolated as the tetra-acetates.[528]



Reagents: i, OsO_4; ii, H^+; iii, $MeCO_3H$; iv, NaOH

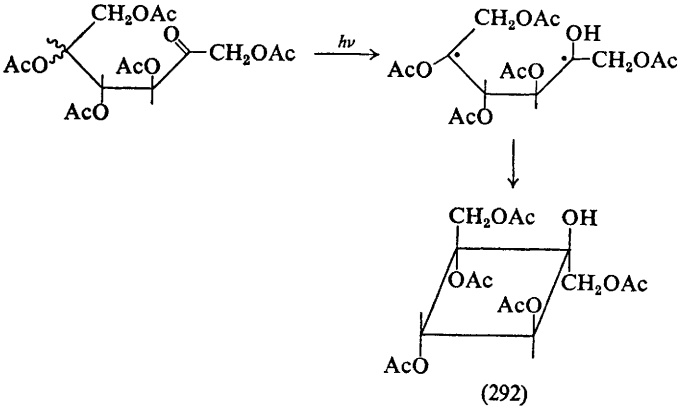
Scheme 113

Asymmetrically substituted *myo*-inositols have been resolved by means of their diastereoisomeric orthoacetates, using a trans-ortho-esterification reaction with 3,4,6-tri-*O*-acetyl-1,2-*O*-ethylorthoacetyl-β-D-mannopyranose.[529] Syntheses of 1- and 4-*O*-benzyl-*myo*-inositol have been reported,[530] and selective benzoylation of 1,2-*O*-cyclohexylidene-*myo*-inositol has been shown to proceed essentially as the corresponding selective toluene-*p*-sulphonylation (see Vol. 6, p. 126).[531]

Eleven stereoisomers of the dianhydroinositols have been prepared from inositol disulphonates, and structures were assigned on the basis of n.m.r. spectroscopy.[532] A study of *C*-methylinositols has indicated that stereoisomers can be characterized by means of mass spectrometry.[533]

[528] C. R. Kowarski and S. Sarel, *J. Org. Chem.*, 1973, **38**, 117.
[529] V. I. Shvets, B. A. Klyashchitskii, A. E. Stepanov, and R. P. Evstigneeva, *Tetrahedron*, 1973, **29**, 331.
[530] B. A. Klyashchitskii, E. B. Krylova, and V. I. Shvets, *Zhur. obshchei Khim.*, 1972, **42**, 2586.
[531] T. Suami, S. Ogawa, K. Ohashi, and S. Oki, *Bull. Chem. Soc. Japan*, 1972, **45**, 3660.
[532] T. Suami, S. Ogawa, and S. Oki, *Chem. Letters*, 1973, 901 (*Chem. Abs.*, 1973, **79**, 115 817v).
[533] A. Buchs and E. Charollais, *Helv. Chim. Acta*, 1973, **56**, 207.

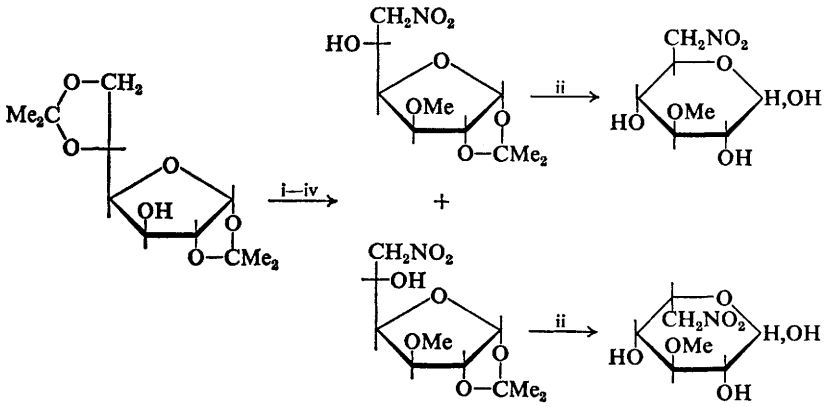
A novel cyclobutane derivative (292) has been obtained in yields of 12 and 16%, respectively, by u.v.-irradiation of either of the C-5-epimers 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose or -L-sorbose.[534] Several derivatives of the parent of (292) were prepared and the conformation of the four-membered ring was discussed. The cyclic product is assumed to be formed by a conventional biradical mechanism (Scheme 114).



(292)

Scheme 114

The epimeric 6-nitro-sugars shown in Scheme 115 have been prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by conventional transformations.[535] Cyclization of these nitro-hexoses by an internal Henry

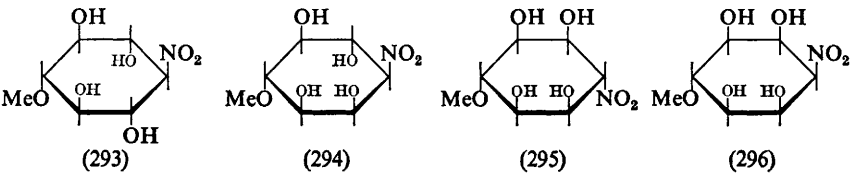


Reagents: i, MeI–BaO; ii, H^+; iii, IO_4^-; iv, $MeNO_2$–MeONa

Scheme 115

[534] R. L. Whistler and L. W. Doner, *J. Org. Chem.*, 1973, **38**, 2900.
[535] J. Kovář and H. H. Baer, *Canad. J. Chem.*, 1973, **51**, 1801.

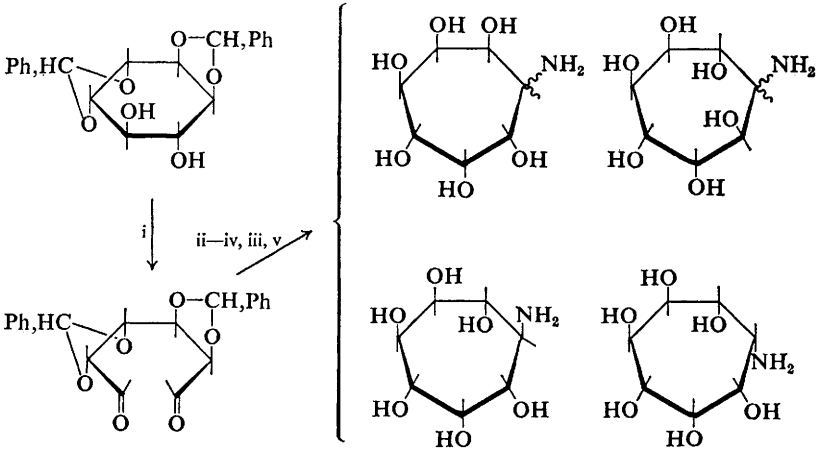
reaction furnished four stereoisomeric nitro-inositol monomethyl ethers possessing the *scyllo* (293), DL-*myo*-1 (294), *muco*-3 (295), and *epi*-3 (296) configurations. Nitro-inositols have also been prepared from the unsaturated, branched-chain sugar (237) by addition of nitryl iodide, reductive dehalogenation of the adduct with sodium borohydride, and cyclization.[466]



2,2-Dimethoxypropane in DMF in the presence of toluene-*p*-sulphonic acid has been used to prepare 1,3-dioxolans from vicinal *cis*- and *trans*-hydroxy-groups in a variety of *N*-acetyl- and *N*-(benzyloxycarbonyl)-aminocyclitols.[203] In one sterically favourable case, an *N,O*-isopropylidene (*i.e.* an *N*-acetyl-2,2-dimethyloxazolidine) derivative (59) was obtained.

A new development in cyclitol chemistry has been the synthesis of aminohexahydroxycycloheptanes (isolated as hydrochlorides) by cyclization of 2,3:4,5-di-*O*-benzylidene-L-*manno*-hexodialdose with nitromethane (Scheme 116).[536]

Addition of vinylmagnesium bromide to the nitro-olefins (297) afforded the corresponding 5-*C*-vinyl derivatives (298), the L-*ido*-configuration of

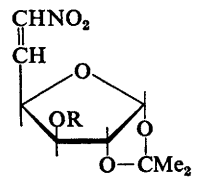


Reagents: i, $Pb(OAc)_4$; ii, $MeNO_2$–MeONa; iii, Ac_2O–py; iv, Ni–H_2; v, HCl

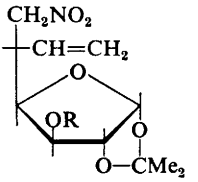
Scheme 116

[536] I. Dyong and R. Bonn, *Chem. Ber.*, 1973, **106**, 944.

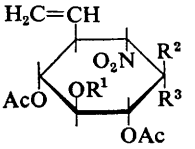
which was established by deacetonation, intramolecular cyclization, and acetylation to afford either of the nitro-cyclitols (299) or (300), depending on the conditions used for cyclization.[537]



(297) R = Ac or Bn



(298)



(299) R^1 = Bn(Ac), R^2 = OAc, R^3 = H
(300) R^1 = Bn(Ac), R^2 = H, R^3 = OAc

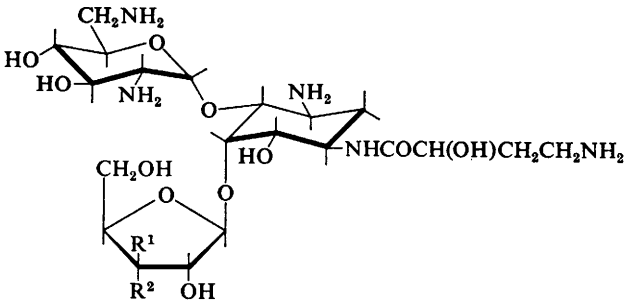
[537] T. Iida, M. Funabashi, and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1973, **46**, 3203.

20
Antibiotics

Amino-glycoside Antibiotics*

Considerable activity has continued in this field during the year under review. A review (in German) has appeared on the synthesis of mono- and di-amino-glycosides of 2-deoxy-streptamine.[49]

A number of papers have dealt with the chemistry of butirosins A (301) and B (302). Antibiotics BU-1709-E_1 and -E_2, minor products from the preparation of butirosins A and B, have been shown to have butirosin-like structures, but with a residue of paromamine in place of one of neamine.[538] The (*S*)-(−)-4-amino-2-hydroxybutyryl side-chain in (301) has been replaced by a wide variety of mono- and poly-functional amino-acids,[539] and the furanosyl linkages in (301) and (302) have been cleaved to give the neamine derivative.[540] A method has been developed for the synthesis of butirosin B (302) from ribostamycin,[541] and 3′,4′-dideoxy-butirosin B has been obtained from the parent antibiotic by means of a Tipson–Cohen



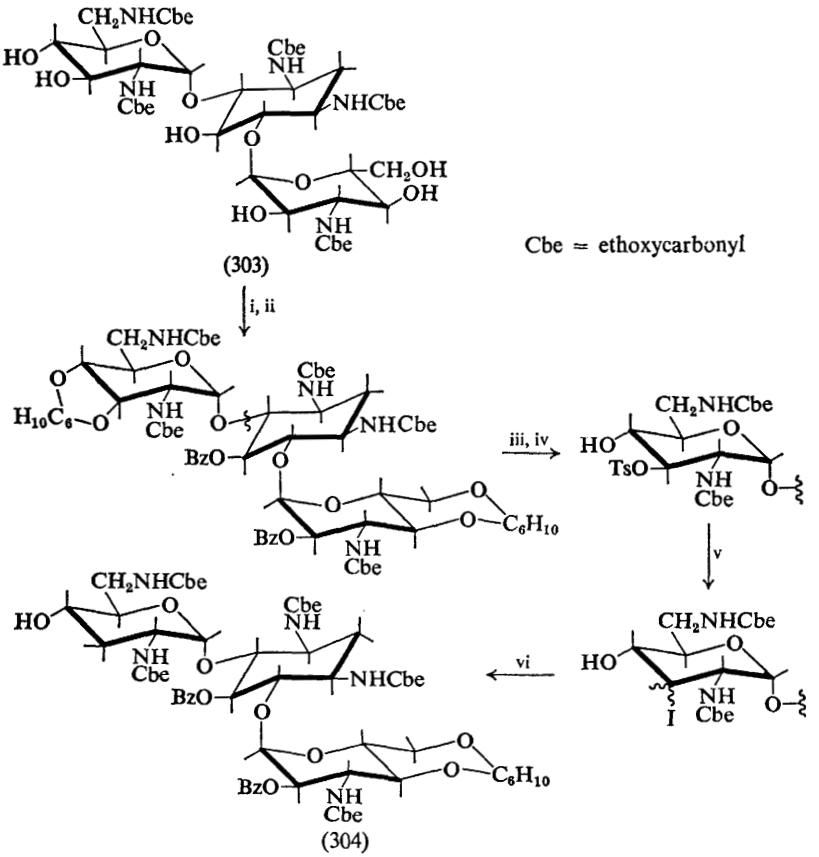
(301) R^1 = OH, R^2 = H (butirosin A)
(302) R^1 = H, R^2 = OH (butirosin B)

[538] H. Tsukiura, K. Saito, S. Kobaru, M. Konishi, and H. Kawaguchi, *J. Antibiotics*, 1973, **26**, 386.
[539] T. H. Haskell, R. Rodebaugh, N. Plessas, D. Watson, and R. D. Westland, *Carbohydrate Res.*, 1973, **28**, 263.
[540] H. Tsukiura, K. Fujisawa, M. Konishi, K. Saito, K. Numata, H. Ishikawa, T. Miyaki, K. Tomita, and H. Kawaguchi, *J. Antibiotics*, 1973, **26**, 351.
[541] E. Akita, Y. Horiuchi, and S. Yasuda, *J. Antibiotics*, 1973, **26**, 365.

* See also Chapter 8.

reaction.[542] Details of syntheses of 3′-deoxy-[543] and 3′,4′-dideoxy-kanamycin B[544] have been published; the key steps in the former synthesis [starting from penta-*N*-ethoxycarbonyl-kanamycin B (303)] leading to the protected product (304) are shown in Scheme 117.

The discovery of the aminohydroxybutyryl side-chain in the butirosins has led to the attachment of this side-chain to N-1 of other amino-glycoside



Reagents: i, $C_6H_{10}(OMe)_2$–DMF–TsOH; ii, BzCl–py; iii, MeOH–H^+; iv, TsCl–py; v, NaI–DMF; vi, H_2–Ni

Scheme 117

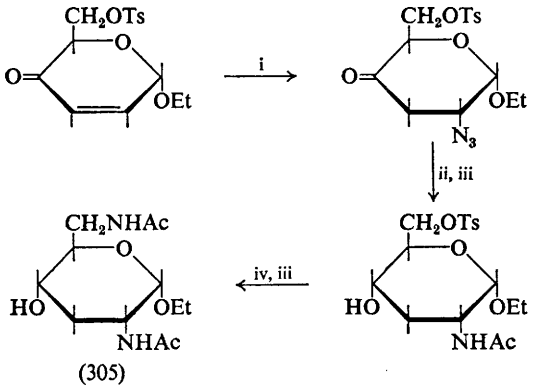
[542] D. Ikeda, T. Tsuchiya, S. Umezawa, H. Umezawa, and M. Hamada, *J. Antibiotics*, 1973, **26**, 307.
[543] Y. Takagi, T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 403.
[544] S. Umezawa, H. Umezawa, Y. Okazaki, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1972, **45**, 3624.

antibiotics; such derivatives of 3′,4′-dideoxy-neamine,[545] kanamycin B,[546] 3′,4′-dideoxy-kanamycin B,[546, 546a] lividomycin A,[547] and BB-K8[548] have been described.

Syntheses of 3′,4′-dideoxy- and 3′,4′,5″-trideoxy-ribostamycin have been reported by Umezawa's group.[549]

Various derivatives of kanamycin A have been examined by mass spectrometry,[550] and a method (based on reaction with 2,4,6-trinitrobenzene sulphonic acid) has been developed for detecting kanamycins in sera and urine.[551] A number of analogues of kanamycin have been prepared using a modified Koenigs–Knorr reaction.[552]

Hexa-*N*-benzyl-neomycin and a number of analogues having the aromatic ring substituted have been obtained by reductive alkylation using the appropriate aryl aldehyde.[553] Ethyl 2,6-diacetamido-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside (305), a derivative of a constituent of nebramycin factor 6, has been synthesized by the route shown in Scheme 118.[554]



(305)

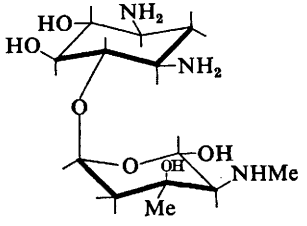
Reagents: i, NaN_3–AcOH; ii, $NaBH_4$; iii, H_2–Pt–C–Ac_2O–MeOH; iv, NaN_3–DMF

Scheme 118

[545] S. Umezawa, D. Ikeda, and T. Tsuchiya, *J. Antibiotics*, 1973, **26**, 304.
[546] S. Kondo, K. Iinuma, H. Yamamoto, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 412.
[546a] S. Kondo, K. Iinuma, H. Yamamoto, Y. Ikeda, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 705.
[547] I. Watanabe, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1973, **26**, 310.
[548] T. Naito, S. Nakagawa, Y. Abe, S. Toda, K. Fujisawa, T. Miyaki, H. Koshiyama, H. Ohkuma, and H. Kawaguchi, *J. Antibiotics*, 1973, **26**, 297.
[549] D. Ikeda, T. Suzuki, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 3210.
[550] D. C. Dejongh, E. B. Hills, J. D. Hribar, S. Hanessian, and T. Chang, *Tetrahedron*, 1973, **29**, 2707.
[551] D. M. Benjamin, J. J. McCormack, and D. W. Gump, *Analyt. Chem.*, 1973, **45**, 1531.
[552] A. Hasegawa, D. Nishimura, and M. Nakajima, *Carbohydrate Res.*, 1973, **30**, 319.
[553] W. T. Shier, S. Ogawa, M. Hichens, and K. L. Rinehart, jun., *J. Antibiotics*, 1973, **26**, 547.
[554] J. Cleophax, S. D. Gero, J. Leboul, and A. Forchioni, *J.C.S. Chem. Comm.*, 1973, 710.

Paromamine and related α-linked disaccharides of streptamine and dihydroconduramine F-4 have been prepared by Koenigs–Knorr reactions,[555] and paromamine has been converted into its 3′-epimer by applying a standard oxidation–reduction sequence.[556] Both 4- and 6-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-2-deoxy-streptamine have resulted from Koenigs–Knorr reactions.[557]

A new pseudo-disaccharide, garamine (306), was produced by treating penta-*N*-benzyloxycarbonyl-sisomicin with an acid ion-exchange resin in THF and then removing the protecting groups.[558]



(306)

The ^{13}C n.m.r. spectra of a number of gentamicins have been tabulated, assigned, and discussed; ^{13}C n.m.r. spectroscopy seems to offer a routine and reliable instrumental method for the elucidation of similar structures.[559] *N*-Salicylidene derivatives of amino-glycoside antibiotics have given easily interpretable fragmentation patterns when examined by mass spectrometry [560] (see also ref. 339).

Products resulting from reaction of nitromethane with the *C*-formyl group of streptomycin have been investigated; the adduct was transformed into a variety of derivatives, whose antibacterial properties were assessed.[561] A procedure based on the periodate–thiobarbituric acid reagent has been devised for the determination of streptomycin.[562]

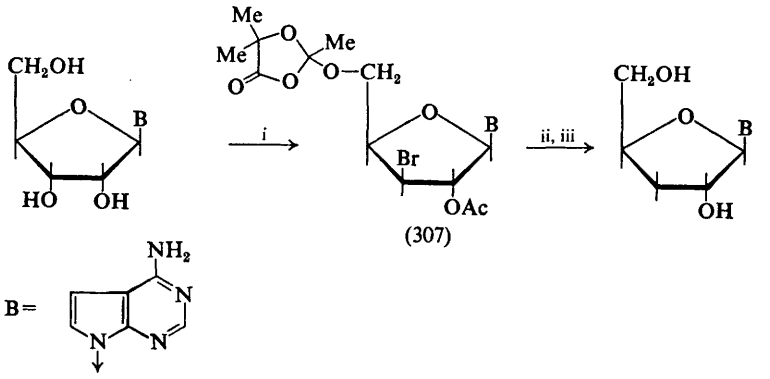
The glycal–nitrosyl chloride procedure for the synthesis of α-glycosides has been applied to the preparation of D-glucosylated derivatives of 2-deoxy-streptamine.[424] Twenty-nine analogues of 2-deoxy-streptamine have been tested to see if they are converted into amino-glycoside antibiotics by 2-deoxy-streptamine-negative mutants, but only a few were transformed into active products.[563]

[555] A. Hasegawa, D. Nishimura, T. Kurokawa, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1773.
[556] S. Hanessian, R. F. Butterworth, and T. Nakagawa, *Carbohydrate Res.*, 1973, **26**, 261.
[557] Y. Nishimura, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 1263.
[558] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J. Antibiotics*, 1973, **26**, 394.
[559] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.
[560] S. Inouye, *Chem. Pharm. Bull.*, 1973, **20**, 2331.
[561] H. Heding, G. J. Fredericks, and O. Lutzen, *Acta Chem. Scand.*, 1972, **26**, 3251.
[562] E. Duda, *Analyt. Biochem.*, 1973, **51**, 651.
[563] W. T. Shier and K. L. Rinehart, jun., *J. Antibiotics*, 1973, **26**, 551.

Nucleoside Antibiotics*

An elegant synthesis of showdomycin [143] (see Scheme 14) and the preparation of a 3′-deoxy-5′-hydroxymethyl analogue of gougerotin [564] have been reported.

2′,3′-Didehydro-2′,3′-dideoxy- [565] and 5′-amino-5′-deoxy-tubercidin [566] have been obtained by standard transformations; treatment of tubercidin with 2-acetoxyisobutyryl chloride or bromide (see also Chapter 7) has provided a route to the 3′-deoxy-compound (Scheme 119).[288] Direct



(307)

Reagents: i, $Me_2C(OAc)COBr$; ii, NH_3; iii, H_2–Pd

Scheme 119

hydrogenolysis of the bromo-compound (307) yielded, *inter alia*, the 2′,3′-dideoxy-derivative, by way of palladium-catalysed *trans*-elimination of the 2-acetoxy-group, while treatment with sodium methoxide gave the 2′,3′-epoxide. Similar reactions were performed on formycin.

^{13}C N.m.r. analyses of formycins A and B and related nucleosides have provided convincing evidence of prototropic tautomerism in the base portion.[567]

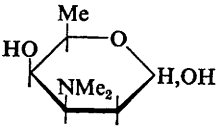
Enzymic methods have been used to incorporate 5-fluorouracil into two 5-fluoropolyoxins.[568]

Macrolide Antibiotics

The structures and absolute stereochemistry of megalomicins A, B, C_1, and C_2 have been elucidated;[569] megalomicin A is comprised of a macrocyclic

[564] T. M. K. Chiu, D. H. Warnock, K. A. Watanabe, and J. J. Fox, *J. Heterocyclic Chem.*, 1973, **10**, 607.
[565] K. Anzai and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 345.
[566] K. Anzai and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 921.
[567] T. R. Krugh, *J. Amer. Chem. Soc.*, 1973, **95**, 4761.
[568] K. Isono, P. F. Crain, T. J. Odiorne, J. A. McCloskey, and R. J. Suhadolnik, *J. Amer. Chem. Soc.*, 1973, **95**, 5788.
[569] R. S. Jaret, A. K. Mallams, and H. Reimann, *J.C.S. Perkin I*, 1973, 1374.

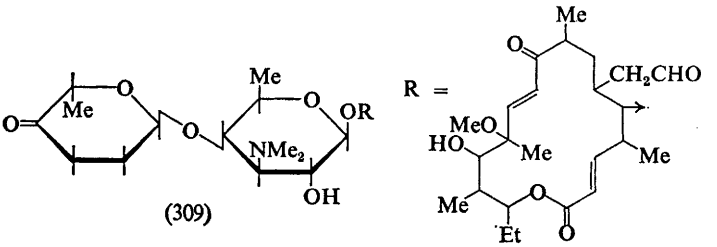
* See also Chapter 21.



(308)

lactone ring bearing D-desosaminyl(3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexopyranosyl), D-rhodosaminyl(2,3,6-trideoxy-3-dimethyl-amino-D-*lyxo*-hexopyranosyl) (308),[570] and L-mycarosyl(2,6-dideoxy-3-*C*-methyl-L-*ribo*-hexopyranosyl) residues. The other members, megalomicins B, C_1, and C_2, are acyl derivatives of megalomicin A. Metabolites of the antibiotic SF-837 have been identified as depropionylated forms.[571]

Antibiotic B-58941 has been found to contain mycaminose and 2,3,6-trideoxy-L-*glycero*-hexopyranos-4-ulose [471] linked as in (309).[572]



(309)

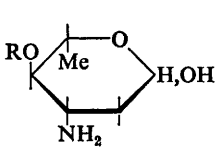
Miscellaneous

Methyl 4-*O*-(α- and β-L-mycarosyl)-β-D-mycosaminides have been synthesized.[573]

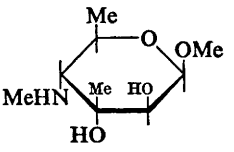
Two new amino-sugars, acosamine (310) and actinosamine (311), have been isolated from actinoidin,[574] and methanolysis of sibiromycin afforded mainly methyl β-sibirosaminide, shown to be methyl 4,6-dideoxy-3-*C*-methyl-4-methylamino-β-D-altropyranoside (312).[575] A partial structure (313) has been assigned to hikosamine, a C_{11}-sugar component of hikizimycin,[576] and further work on the structure of vancomycin has revealed that vancosamine (Vol. 6, p. 135) is linked to position 2 of the D-glucose residue, which is bonded in turn to a complex, aromatic aglycone.[577]

[570] A. K. Mallams, *J.C.S. Perkin I*, 1973, 1369.
[571] S. Inouye, T. Shomura, T. Tsuruoaka, S. Omoto, T. Niida, and K. Umemura, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2366.
[572] T. Suzuki, *Chem. Letters*, 1973, 799 (*Chem. Abs.*, 1973, **79**, 126 741s).
[573] S. Koto, K. Yago, S. Zen, and S. Omura, *Chem. Letters*, 1972, 1091 (*Chem. Abs.*, 1973, **79**, 30 141z).
[574] N. N. Lomakina, I. A. Spiridonova, Yu. N. Sheinker, and T. F. Vlasova, *Khim. prirod. Soedinenii*, 1973, **9**, 101.
[575] A. S. Mesentsev and V. V. Kuljaeva, *Tetrahedron Letters*, 1973, 2225.
[576] K. Uchida and B. C. Das, *Biochemie*, 1973, **55**, 635.
[577] P. J. Roberts, O. Kenward, K. A. Smith, and D. H. Williams, *J.C.S. Chem. Comm.*, 1973, 772.

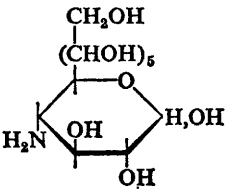
Partial hydrolysis of the antibiotic moenomycin A with trifluoroacetic acid, followed by acetylation and chromatography of the products, furnished, *inter alia*, a disaccharide derivative characterized as (314).[578]



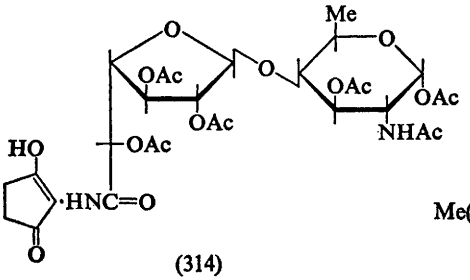
(310) R = H
(311) R = Me



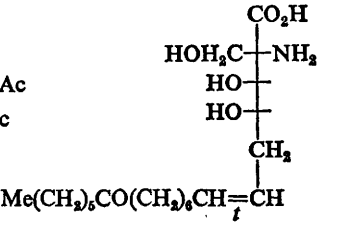
(312)



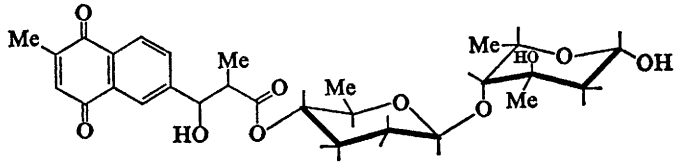
(313)



(314)



(315)



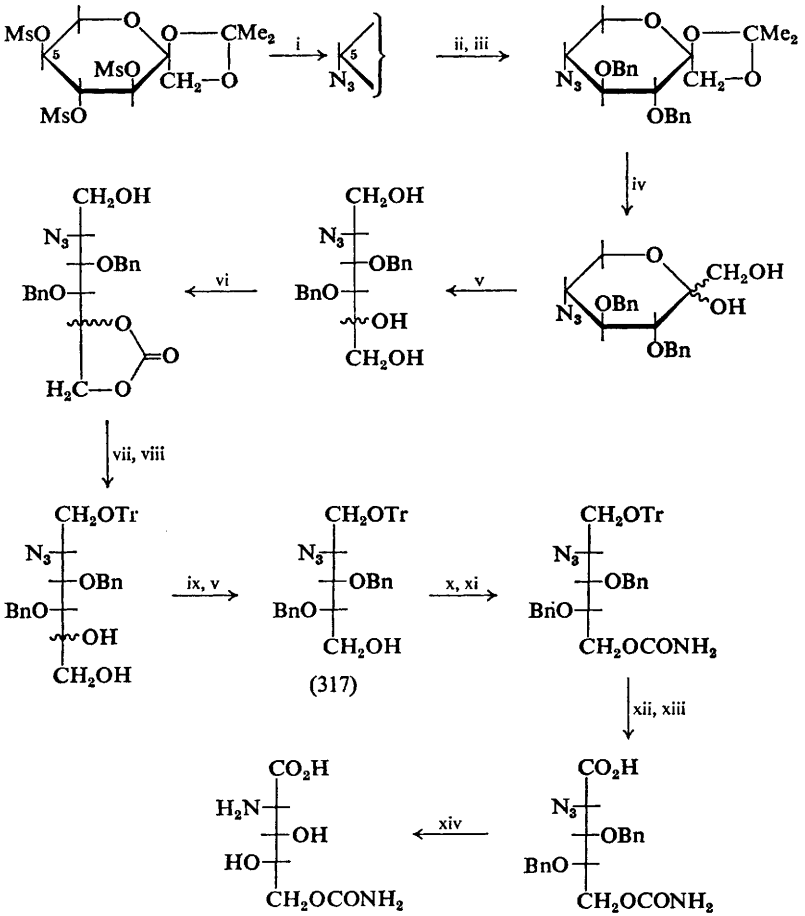
(316)

The structure (315) (or the enantiomer) has been assigned to the antifungal compound 'myriocin' (from *Myriococcum albomyces*),[579] and the disaccharide ester (316), containing amicetose and a new sugar, 2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexose, was obtained on hydrolysis of axenomycin B.[580]

Streptolydigin, an acyltetramic acid antibiotic that inhibits RNA polymerase, has been shown to be a *N*-β-L-rhodinosyl (2,3,6-trideoxy-β-L-*threo*-hexopyranosyl) derivative of tirandamycin containing the sugar bound by a glycosylaminyl linkage.[581]

[578] P. Welzel, H. Buhlke, P. Michalke, J. Simons, L. Winterfeld, and R. Tschesche, *Tetrahedron Letters*, 1973, 227.
[579] J. F. Bagli, D. Kluepfel, and M. St.-Jacques, *J. Org. Chem.*, 1973, **38**, 1253.
[580] F. Arcamone, W. Barbieri, G. Franceschi, S. Penco, and A. Vigevani, *J. Amer. Chem. Soc.*, 1973, **95**, 2008.
[581] D. J. Duchamp, A. R. Branfman, A. C. Button, and K. L. Rinehart, jun., *J. Amer. Chem. Soc.*, 1973, **95**, 4077.

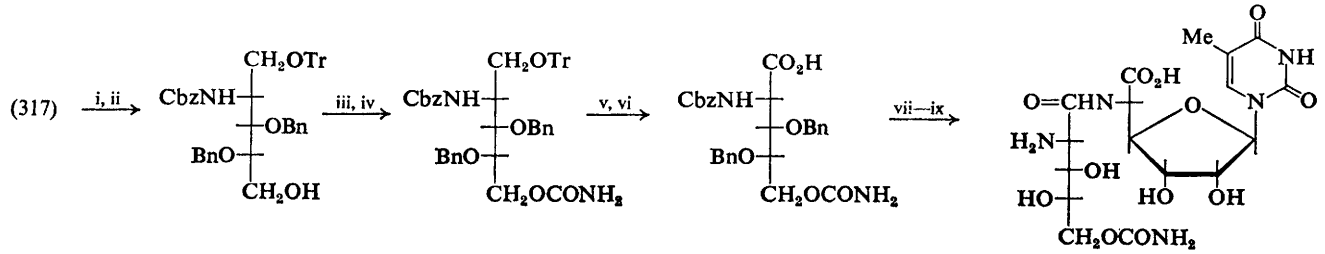
Synthetic 6-de-(1-hydroxyethyl)- and 7-*O*-methyl-lincomycins showed reduced and comparable activities, respectively, in relation to lincomycin,[582] whereas modification of the carbohydrate moiety, to produce D-*gluco*- or D-*ido*-analogues, destroyed any activity.[583] 2-*O*-Methyl- and 2-deoxy-lincomycins were also synthesized, but they showed only marginal activity compared with the parent antibiotic.[584] 2-Amino-5-*O*-carbamoyl-2-deoxy-



Reagents: i, NaN_3; ii, KOH; iii, BnCl; iv, H^+; v, $NaBH_4$; vi, $COCl_2$–py; vii, TrCl; viii, NaOMe; ix, $NaIO_4$; x, $pNO_2C_6H_4OCOCl$; xi, NH_3; xii, CF_3CO_2H; xiii, CrO_3–H_2SO_4; xiv, H_2–Pd–C

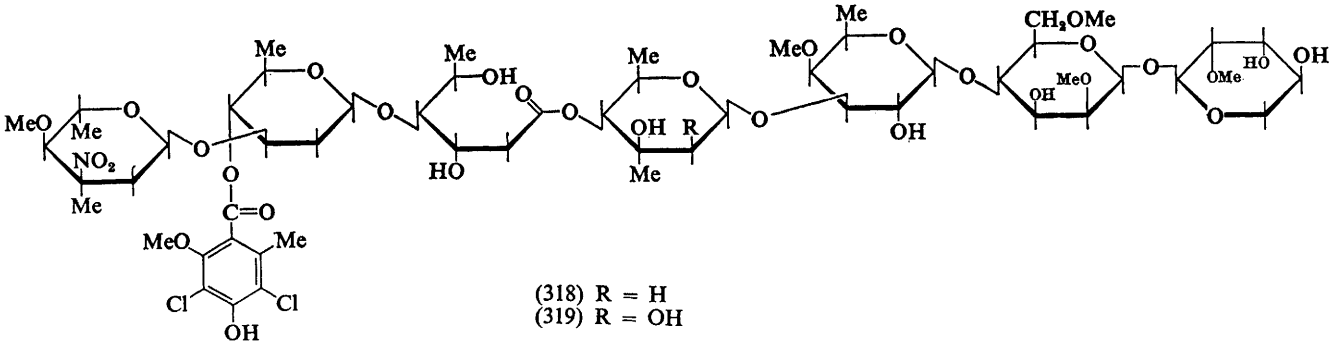
Scheme 120

[582] B. Bannister, *J.C.S. Perkin I*, 1973, 1676.
[583] B. Bannister, *J.C.S. Perkin I*, 1972, 3031.
[584] B. Bannister, *J.C.S. Perkin I*, 1972, 3025.



Reagents: i, $LiAlH_4$; ii, CbzCl; iii, $pNO_2C_6H_4OCOCl$; iv, NH_3; v, AcOH; vi, CrO_3–H_2SO_4; vii, *N*-hydroxysuccinimide–DCC; viii, (279) (see p. 133); ix, H_2–Pd–C

Scheme 121



(318) R = H
(319) R = OH

L-xylonic acid (5-*O*-carbamoyl-polyoxamic acid), a component of polyoxin A, has been synthesized by the route shown in Scheme 120.[487] One of the compounds (317) obtained in this synthesis was also used in a synthesis of polyoxin J (Scheme 121).[504]

Structural studies have now been completed on the heptasaccharides everheptose (318) and everheptose B (319) obtained on aqueous acid hydrolysis of everninomicins D and B, respectively.[585, 586] Everheptose B was found to contain a new sugar, D-evalose, the structure and absolute stereochemistry of which were established as 6-deoxy-3-*C*-methyl-D-mannose. The structures of these heptasaccharides are undoubtedly among the most formidable carbohydrate structures to be elucidated.

Syntheses of derivatives of the antibiotic sugars tolyposamine and forosamine have been mentioned in Chapter 8.

[585] A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1973, **95**, 944.

[586] A. K. Ganguly and A. K. Saksena, *J.C.S. Chem. Comm.*, 1973, 531.

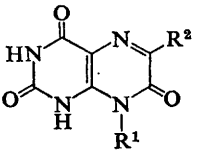
21
Nucleosides

Reviews have appeared on the synthesis of pyrimidine nucleosides (in Russian)[587] and on the conformations of nucleosides (in Polish).[588] A short literature survey of the synthesis of disaccharide nucleosides is contained in a paper describing syntheses of cellobiosyl- and lactosyl-benzimidazoles;[589] the *O*-glycosidic bonds in these compounds can be cleaved selectively to give the corresponding D-glucosylbenzimidazoles.

The first naturally occurring halogen-containing nucleoside, 5-chlorocytidine, has been identified in salmon-sperm DNA; another compound isolated appeared to be 5-chloro-2′-deoxycytidine.[590] Three pteridine derivatives from the mycelium of *P. phosphorium* have been identified as (320)—(322),[591] and the oxathian 'nucleosides' (323) and (324) have been synthesized.[592]

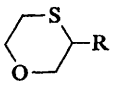
Synthesis

Syntheses have been described of β-D-ribofuranosyl and/or 2-deoxy-β-D-*erythro*-pentofuranosyl derivatives of 1,2,4-triazoles (related to vira-

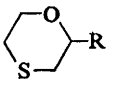


(320) R^2 = H
(321) R^2 = Me
(322) R^2 = $CH(OH)CH_2OH$

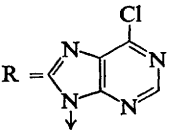
R^1 = D-ribityl



(323)



(324)



[587] B. N. Stepanenko, E. M. Kaz'mina, and Z. S. Dubinkina, *Uspekhi Khim.*, 1973, **42**, 1121.
[588] A. Rabczenko, *Postepy Biochem.*, 1973, **19**, 195.
[589] D. R. Rao and L. M. Lerner, *J. Org. Chem.*, 1972, **37**, 3741.
[590] A. W. Lis, R. K. McLaughlin, D. I. McLaughlin, G. D. Daves, and W. R. Anderson, *J. Amer. Chem. Soc.*, 1973, **95**, 5789.
[591] S. Matsuur, M. Odaka, T. Sugimoto, and T. Goto, *Chem. Letters*, 1973, 343 (*Chem. Abs.*, 1973, **79**, 5523p).
[592] D. M. Vyas and W. A. Szarek, *Carbohydrate Res.*, 1973, **30**, 225.

zole),[593, 593a] pyridones,[594, 595] 1,2-dihydro-2-oxopyrazine 4-oxide,[596] 6-substituted uracils,[597] imidazo[4,5-b]pyrazine and its 4-*N*-oxide (nebularine analogues),[598] 6-chloropurine (an improved synthesis),[599] various pyrido-[2,3-d]pyrimidines (as analogues of sangivamycin),[600] and isoguanine.[601] The structure of a new cytokinin from *Populus robusta* has been confirmed by synthesis as 6-(*o*-hydroxybenzylamino)-9-β-D-ribofuranosylpurine,[602] and a number of nucleosides related to cytokinin have been synthesized.[603]

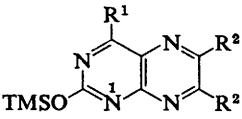
The silyl procedure has been used to prepare 8-bromo-3-glycosyladenines,[604] 8-glycosyl-7-oxo-7,8-dihydropteridines,[605] nucleosides of lumazine,[606] isopterin,[607] and 2-thiopyrimidine (related to rare nucleosides from t-RNA),[608] and 3-oxa-2′-deoxyuridine.[609]

The fusion route to nucleosides has been studied further, and nitrophenols, tolylsulphonamides, and aromatic carboxylic acids have been examined as condensing agents; it was suggested, in the case of theophylline, that some of these agents form molecular complexes with the base.[610] In the synthesis of guanine nucleosides by fusion of N^2-acetylguanine with a number of peracylated aldoses, it was found that the ratio (45 : 55) of 7- and 9-isomers formed is independent of the sugar used, whereas the proportion of anomers is not;[611] the product distribution is apparently determined by the thermodynamic stabilities. 1-Glycosylindazoles have been obtained by fusing peracetylated sugars with indazoles in the presence of iodine, whereas earlier methods, employing either the trimethylsilyl or mercuric cyanide routes, give 2-glycosylindazoles.[612]

[593] J. T. Witkowski, R. K. Robins, G. P. Khare, and R. W. Sidwell, *J. Medicin. Chem.*, 1973, **16**, 935.
[593a] J. T. Witkowski, R. K. Robins, R. W. Sidwell, and L. N. Simon, *J. Medicin. Chem.*, 1972, **15**, 1150.
[594] S. Nesnow, T. Miyazaki, T. Khwaja, R. B. Meyer, and C. Heidelberger, *J. Medicin. Chem.*, 1973, **16**, 524.
[595] R. L. Shone, *Tetrahedron Letters*, 1973, 3079.
[596] P. T. Berkowitz, T. J. Bardos, and A. Bloch, *J. Medicin. Chem.*, 1973, **16**, 183.
[597] R. S. Klein and J. J. Fox, *J. Org. Chem.*, 1972, **37**, 4381.
[598] R. A. Sharma, M. Bobek, F. E. Cole, and A. Bloch, *J. Medicin. Chem.*, 1973, **16**, 643.
[599] H. Guilford, P. O. Larsson, and K. Mosbach, *Chem. Sci.*, 1972, **2**, 165 (*Chem. Abs.*, 1973, **78**, 43 900w).
[600] B. H. Rizkalla and A. D. Broom, *J. Org. Chem.*, 1972, **37**, 3980.
[601] C. L. Schmidt and L. B. Townsend, *J. Heterocyclic Chem.*, 1973, **10**, 687.
[602] R. Horgan, E. W. Hewett, J. G. Purse, and P. J. Wareing, *Tetrahedron Letters*, 1973, 2827.
[603] M. J. Robins and E. M. Trip, *Biochemistry*, 1973, **12**, 2179.
[604] C. G. Tindall, R. K. Robins, R. L. Tolman, and W. Hutzenlaub, *J. Org. Chem.*, 1972, **37**, 3985.
[605] W. Pfleiderer, D. Autenrieth, and M. Schranner, *Chem. Ber.*, 1973, **106**, 317.
[606] G. Ritzmann and W. Pfleiderer, *Chem. Ber.*, 1973, **106**, 1401.
[607] K. Harzer and W. Pfleiderer, *Helv. Chim. Acta*, 1973, **56**, 1225.
[608] H. Vorbrüggen and P. Strehlke, *Chem. Ber.*, 1973, **106**, 3039.
[609] M. Bobek, A. Bloch, and S. Kuhar, *Tetrahedron Letters*, 1973, 3493.
[610] M. Sekiya, T. Yoshino, H. Tanaka, and Y. Ishido, *Bull. Chem. Soc. Japan*, 1973, **46**, 556.
[611] H. Iwamura, M. Miyakado, and T. Hashizume, *Carbohydrate Res.*, 1973, **27**, 149.
[612] I. A. Korbukh, F. F. Blanco, and M. N. Preobrazhenskaya, *Tetrahedron Letters*, 1973, 4619.

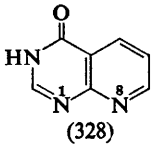
Iodine was also used as catalyst in the synthesis of 3-cyano-1-glycosylindazoles by a fusion procedure.[613]

The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the bases (325)—(327) gave lumazine and isopterin nucleosides linked

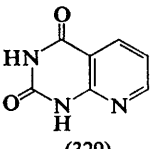


(325) R^1 = OTMS, R^2 = H
(326) R^1 = NHTMS, R^2 = H
(327) R^1 = NHTMS, R^2 = Me

TMS = $-SiMe_3$



(328)



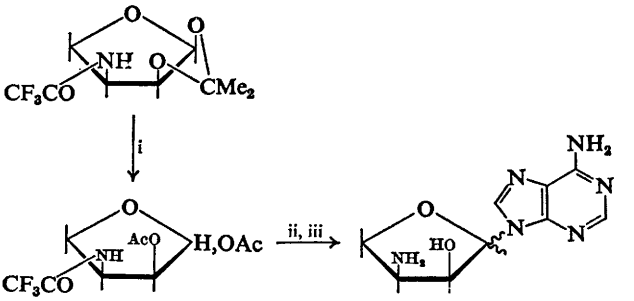
(329)

through N-1; a C-1′–N-1 *syn-anti* isomeric pair was found to be stable at room temperature.[614] D-Ribosylation of (328) occurred only at N-1 whereas in the oxy-compound (329), it occurred at both N-1 and N-8; compounds substituted at N-8 isomerized to N-1 derivatives in the presence of aluminium trichloride.[615] Bis(theophyllin-7-yl)mercury has been used in an efficient synthesis of theophylline nucleosides, with yields in the range 70—85%.[616]

Syntheses of a number of 9-glycosyladenines have been described, including 9-(α- and β-D-xylofuranosyl)adenine.[214] Lerner has synthesized 9-(6-deoxy-α-L-mannofuranosyl)adenine by unambiguous routes, and has shown that it differs from the compound previously assigned this structure.[617] Attempts to prepare this compound following acetolysis of 1,5-di-*O*-acetyl-6-deoxy-2,3-*O*-isopropylidene-L-mannofuranose gave 9-(6-deoxy-α-L-mannopyranosyl)- and 9-(6-deoxy-β-L-glucofuranosyl)-adenines, in addition to the desired product, ring expansion and epimerization at C-2 having occurred on acetolysis. A synthesis of 9-(3-amino-3-deoxy-β-L-erythrofuranosyl)adenine is shown in Scheme 122.[618]

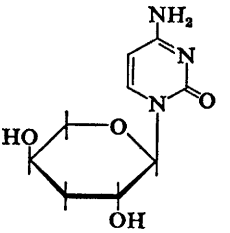
Pentopyranine C (330) and its furanosyl analogue have been obtained from the appropriate 3-deoxy-L-*threo*-pentose triacetates,[619] and a number

[613] I. A. Korbukh, F. F. Blanco, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1973, **9**, 852.
[614] W. Pfleiderer, G. Ritzmann, K. Harzer, and J. C. Jochims, *Chem. Ber.*, 1973, **106**, 2982.
[615] B. H. Rizkalla, A. D. Broom, M. G. Stout, and R. K. Robins, *J. Org. Chem.*, 1972, **37**, 3975.
[616] A. J. Freestone, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1973, **28**, 378.
[617] L. M. Lerner, *J. Org. Chem.*, 1973, **38**, 3704.
[618] J. M. J. Tronchet and R. Graf, *Helv. Chim. Acta*, 1973, **56**, 2689.
[619] T. M. K. Chiu, H. Ohrui, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1973, **38**, 3622.



Reagents: i, AcOH–Ac_2O–H^+; ii, $C_5H_4N_5HgCl$; iii, MeONa–MeOH

Scheme 122

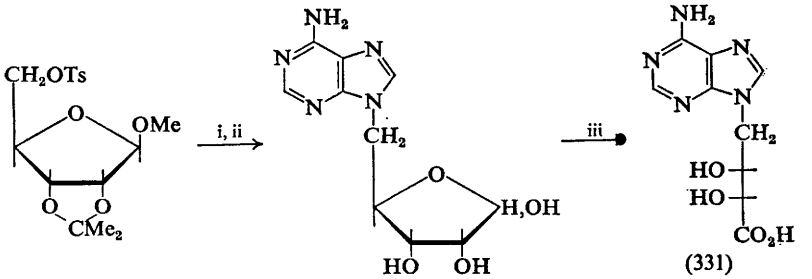


(330)

of riboflavin analogues, with different substituents on the aromatic ring, have been synthesized.[620]

'Reversed' Nucleosides

A synthesis of 'reversed' adenine is shown in Scheme 123. When this nucleoside was degraded in oxygenated dilute alkali, the sodium salt of



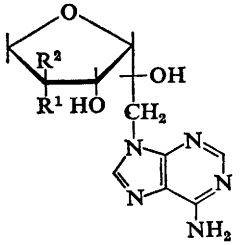
(331)

Reagents: i, $C_5H_4N_5Na$; ii, H^+; iii, O_2–NaOH

Scheme 123

[620] J. P. Lambooy and J. P. Lambooy, *J. Medicin. Chem.*, 1973, **16**, 765.

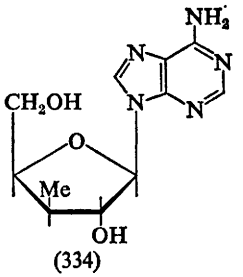
eritadenine (331) (a hypocholesterolemic compound) was obtained; eritadenine was similarly obtained from the corresponding 'reversed' D-arabinosyl nucleoside.[621] The foregoing work was extended to produce adenines with the following substituents at N-9: (5-deoxy-D-arabinonic acid)-5-yl, (5-deoxy-D-ribonic acid)-5-yl, (4-deoxy-D-threonic acid)-4-yl, and (4-deoxy-L-erythronic acid)-4-yl.[622] Condensation of several ω-deoxy-ω-iodo-sugars with pyrimidine and purine bases afforded a number of 'reversed' nucleosides containing both D-ribofuranosyl and D-glucopyranosyl residues.[623] The '1′-deoxy-reversed' nucleosides (332) and (333) were obtained from the appropriate sulphonates by displacement with the sodium salt of adenine.[624]



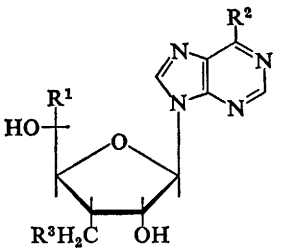
(332) R^1 = OH, R^2 = H
(333) R^1 = H, R^2 = OH

Nucleosides with Branched-chain Components

A number of branched-chain sugars described in Chapter 15 have been utilized in syntheses of the nucleosides (334),[465] (234) (see Scheme 96), (335),[464a] and (336).[464]



(334)

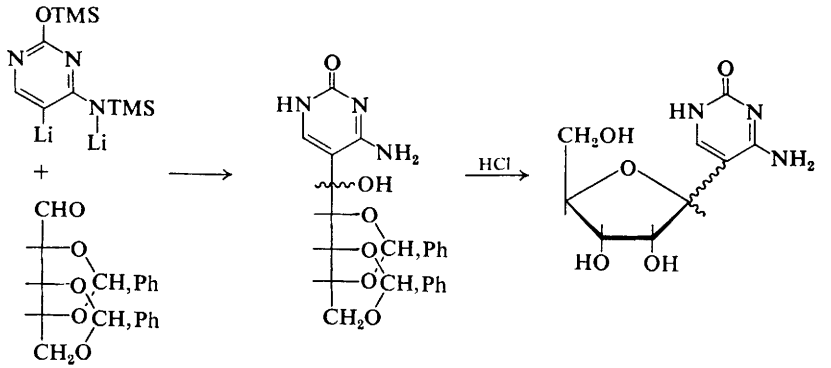


(335) R^1 = H, R^2 = NMe_2, R^3 = $CONH_2$
(336) R^1 = CH_2OH, R^2 = Cl, R^3 = CN

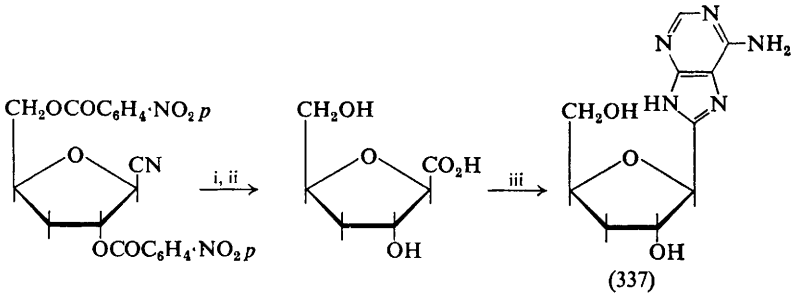
[621] M. Kawazu, T. Kanno, S. Yamamura, T. Mizoguchi, and S. Saito, *J. Org. Chem.*, 1973, **38**, 2887.
[622] N. Takamura, N. Taga, T. Kanno, and M. Kawazu, *J. Org. Chem.*, 1973, **38**, 2891.
[623] S. Fukatsu, Y. Takeda, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 3165.
[624] G. Giovanninetti, L. Nobile, A. Andreani, A. Ferranti, M. Amorosa, and J. Defaye, *Carbohydrate Res.*, 1973, **27**, 243.

C-Nucleosides

A synthesis of α- and β-*pseudo*-cytidines is shown in Scheme 124; assignments of configuration were based on deamination experiments to give the known *pseudo*-uridines.[625] Glycosyl nitriles have been used in the elaboration of C-8-linked purine nucleosides;[626] an interesting example is the synthesis of the cordycepin analogue (337) shown in Scheme 125.[627]



Scheme 124



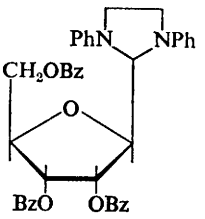
Reagents: i, H^+; ii, ^-OH; iii, 4,5,6-triaminopyrimidine

Scheme 125

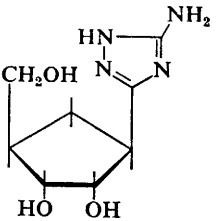
A glycosyl nitrile was also used to prepare the imidazolidine (338) (see Scheme 15),[144] and the glycosyl barbiturate (34) was obtained *via* the related glycosyl malonate.[147]

A synthesis of the DL-form of the *C*-nucleoside (339), containing a carbocyclic ring in place of the D-ribofuranosyl ring, has been described.[628]

[625] S. David and A. Lubineau, *Carbohydrate Res.*, 1973, **29**, 15.
[626] A. Kolb, C. Gouyette, H. D. Tam, and J. Igolen, *Tetrahedron Letters*, 1973, 2971.
[627] H. S. El Khadem and E. S. H. El Ashry, *Carbohydrate Res.*, 1973, **29**, 525.
[628] G. Just and G. Reader, *Tetrahedron Letters*, 1973, 1525.



(338)

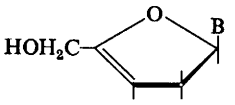


(339)

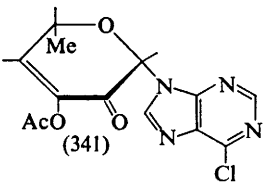
2,2′-(D-*manno*-Tetritol-1,4-diyl)bisbenzimidazole has been prepared and tested for anti-tumour activity.[629]

Unsaturated Nucleosides

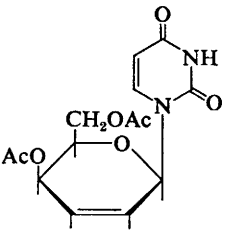
A number of unsaturated nucleosides are referred to in Chapter 14. Elimination of substituents (often β to a carbonyl group) has been used in the synthesis of nucleosides (340) (see Scheme 90) [446] and (341).[630] Interest



(340)



(341)

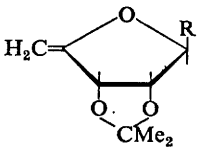


(342)

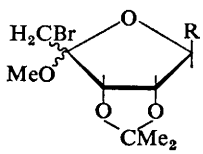
has continued in the fusion of acylated glycals with bases as a means of synthesizing 2′,3′-unsaturated nucleosides; glycal derivative with the base bonded at C-3′ were also formed.[431, 631] The uracil nucleoside (342) obtained in this way was used in a synthesis of cytosinine, the nucleoside component of blasticidin S.[632]

The unsaturated nucleosides (343) and (344) reacted with *N*-bromosucciminide in methanol to give the expected addition products (345) and (346),[633, 633a] but more complex reactions occurred in aqueous solution. The uracil derivative (343) gave a 2,4′-cyclonucleoside (347), whereas the adenine derivative (344) underwent more extensive reaction to give (348) and (349), the latter presumably arising *via* a N^3,4′-cyclonucleoside intermediate.

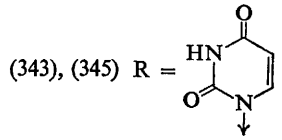
[629] A. J. Charlson, *Carbohydrate Res.*, 1973, **28**, 118.
[630] K. Antonakis and M. Bessodes, *Carbohydrate Res.*, 1973, **30**, 192.
[631] G. Alonso, M. Fuertes, G. García-Muñoz, R. Madroñero, and M. Stud, *J. Medicin. Chem.*, 1973, **16**, 1056.
[632] T. Kondo, H. Nakai, and T. Goto, *Tetrahedron*, 1973, **29**, 1801.
[633] T. Sasaki, K. Minamoto, S. Kuroyanagi, and K. Hattori, *Tetrahedron Letters*, 1973, 2731.
[633a] T. Sasaki, K. Minamoto, and K. Hattori, *J. Amer. Chem. Soc.*, 1973, **95**, 1350.

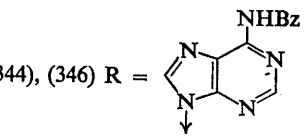


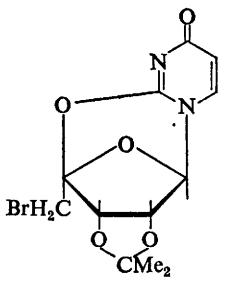
(343), (344)



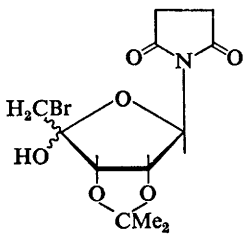
(345), (346)



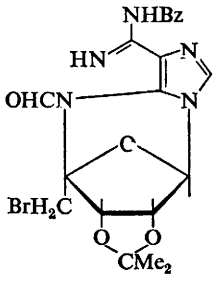




(347)



(348)

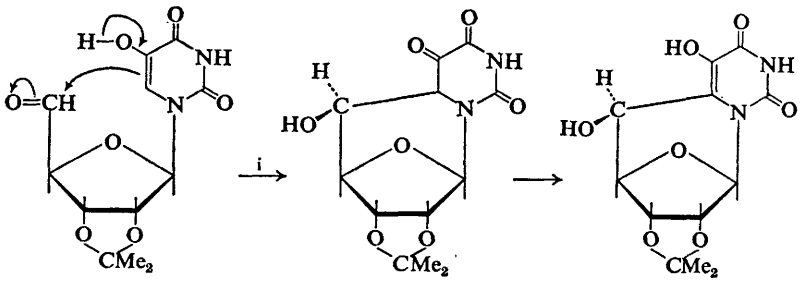


(349)

Cyclonucleosides

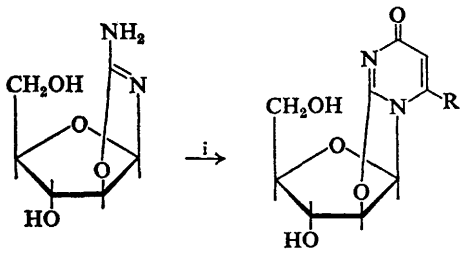
Phosphorus oxychloride in aqueous ethyl acetate has been used to convert cytidine derivatives into 2,2′-cyclonucleosides.[634] Both 2′- and 3′-deoxyinosines have been synthesized *via* 8,2′- and 8,3′-thioanhydroinosines.[635] The key step in the synthesis of a cyclonucleoside containing a direct C-6–C-5′ bond is shown in Scheme 126.[636] Two more examples have been reported of the reaction of acetylenic esters with oxazolines to give 2,2′-cyclonucleosides of pyrimidines (Scheme 127); further reactions gave 1-β-D-arabinofuranosyluracil in one case,[637] and, in the other, 6-methyl-2′-deoxyuridine was obtained by way of a 2′-chloro-derivative.[638]

[634] T. Kanai, M. Ichino, A. Hoshi, F. Kanzawa, and K. Kuretani, *J. Medicin. Chem.*, 1972, **15**, 1218.
[635] A. Yamazaki, M. Akiyama, I. Kumashiro, and M. Ikehara, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1143.
[636] J. A. Rabi and J. J. Fox, *J. Org. Chem.*, 1972, **37**, 3898.
[637] D. H. Shannahoff and R. A. Sanchez, *J. Org. Chem.*, 1973, **38**, 593.
[638] A. Holý, *Tetrahedron Letters*, 1973, 1147.



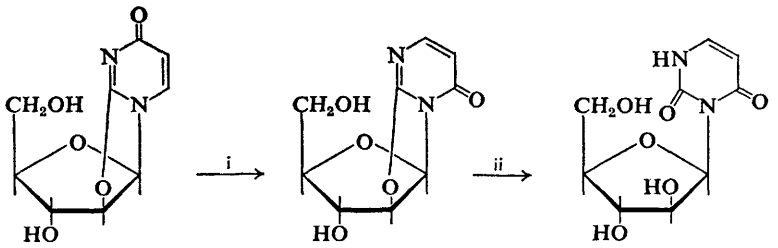
Reagent: i, $NaHCO_3$–H_2O

Scheme 126



Reagent: i, $RC{\equiv}CCO_2Et(Me)$ (R = H or Me)

Scheme 127

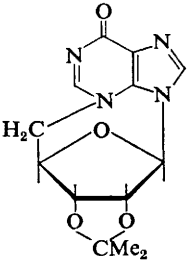


Reagents: i, HF; ii, NaOH

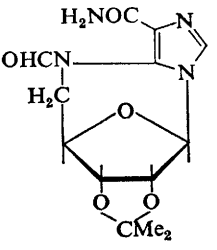
Scheme 128

2,2′-Anhydrouridine rearranged in liquid hydrogen fluoride to give a new cyclonucleoside, which opened in alkali to yield the D-*arabino*-isomer of isouridine (see Scheme 128).[639] The cyclonucleoside (350) was prepared from the corresponding 5′-toluene-*p*-sulphonate, and, in aqueous solution, it was readily hydrolysed to (351), which previously had been reported to have the structure (350).[640]

[639] J. O. Polazzi and M. P. Kotick, *Tetrahedron Letters*, 1973, 2939.
[640] J. T. Witkowski, G. P. Kreishman, M. P. Schweizer, and R. K. Robins, *J. Org. Chem.*, 1973, **38**, 180.



(350)

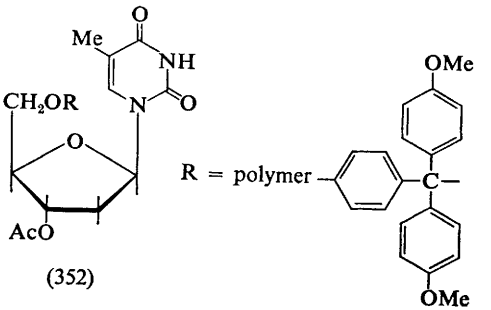


(351)

Derivatives

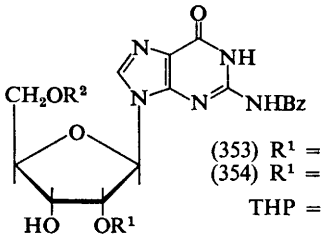
Alkylation of cytidine and suitable derivatives in strongly alkaline media gave rise to mono-, di-, and tri-*O*-alkyl derivatives, which can be converted into the corresponding uridine derivatives on deamination.[641] The 2′-, 3′-, and 5′-*O*-benzyl ethers of cytidine [642] and the 2′-*O*-methyl ethers of 1-β-D-arabinofuranosylcytosine and uracil [643] have been prepared by standard procedures. Indirect methods have also been employed; for example, 1- and 3-(2′-*O*-methyl-β-D-ribofuranosyl)uracils were prepared from 2-*O*-methyl-D-ribose,[644] and other 2′-*O*-alkyl nucleosides were obtained by alkylation of nucleoside 3′,5′-cyclic phosphates under alkaline conditions and subsequent dephosphorylation.[645]

Jones' studies of 3′-*O*-carboxymethyl derivatives of nucleosides (nucleotide analogues) have now been extended to include deoxynucleosides,[646] and the resin-bonded thymidine derivative (352) has been used in solid-support syntheses of polynucleotides.[647] The guanosine derivatives (353)



(352)

[641] J. T. Kusmierek, J. Giziewicz, and D. Shugar, *Biochemistry*, 1973, **12**, 194.
[642] W. Hutzenlaub and W. Pfleiderer, *Chem. Ber.*, 1973, **106**, 665.
[643] J. Giziewicz and D. Shugar, *Acta Biochim. Polon.*, 1973, **20**, 73.
[644] A. H. Haines, *Tetrahedron*, 1973, **29**, 2807.
[645] I. Tazawa, S. Tazawa, J. L. Alderfer, and P. O. P. Ts'o, *Biochemistry*, 1972, **11**, 4931.
[646] M. D. Edge, A. Hodgson, A. S. Jones, M. MacCoss, and R. T. Walker, *J.C.S. Perkin I*, 1973, 290.
[647] H. Koster and F. Cramer, *Annalen*, 1972, **766**, 6.

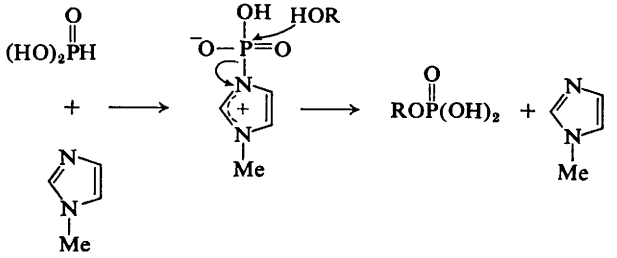


(353) R^1 = THP, R^2 = H
(354) R^1 = THP, R^2 = $COCH_2OTr$
THP = tetrahydropyranyl

and (354) have been obtained by standard methods for use in syntheses of oligoribonucleotides.[648]

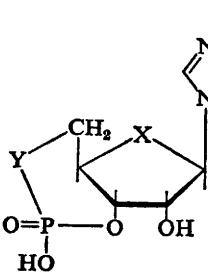
Methods for trimethylsilylation of nucleosides and nucleotides that result in reaction either at the hydroxy-groups of the sugar moiety, or at the purine or pyrimidine base as well as at the hydroxy-groups of the sugar moiety, have been evaluated.[649] Two papers by Ogilvie have described the usefulness of the t-butyldimethylsilyl ether as a protecting group in the synthesis of nucleosides; these ethers are most readily formed at the 5′-position, and can be removed by treatment with tetra-n-butylammonium fluoride in THF, a reagent unlikely to affect other protecting groups commonly used.[172, 650]

Nucleosides have been phosphorylated with phosphorous acid in the presence of mercuric chloride and *N*-methylimidazole, the effective reagent being an imidazolium phosphate (see Scheme 129).[651] The 5′-phosphates of several 8,2′-thioanhydropurine nucleosides have been described,[652] as have the isomeric, sulphur-containing 3′,5′-cyclic phosphates (355)[653] and (356);[654] the latter was prepared by way of a displacement with thiophosphate on 5′-deoxy-5′-iodoadenine. A 3′,5′-cyclic phosphoramidate (358)



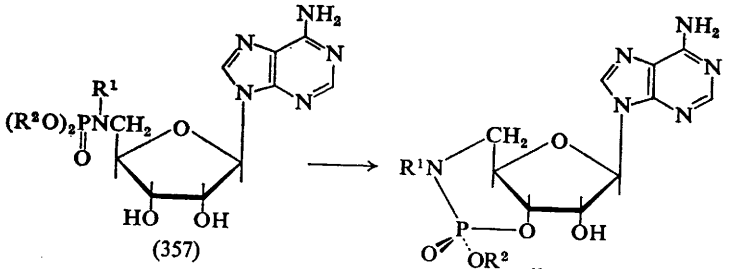
Scheme 129

[648] T. Neilson, E. V. Wastrodowski, and E. S. Werstiuk, *Canad. J. Chem.*, 1973, **51**, 1068.
[649] L. Pichat, J. Godbillon, and M. Herbert, *Bull. Soc. chim. France*, 1973, 2709.
[650] K. K. Ogilvie, *Canad. J. Chem.*, 1973, **51**, 3799.
[651] H. Takaku, Y. Shimada, and H. Oka, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1845.
[652] K. K. Ogilvie and L. A. Slotin, *Canad. J. Chem.*, 1973, **51**, 2397.
[653] A. K. M. Anisuzzaman, W. C. Lake, and R. L. Whistler, *Biochemistry*, 1973, **12**, 2041.
[654] D. A. Shuman, J. P. Miller, M. B. Scholten, N. L. Simon, and R. K. Robins, *Biochemistry*, 1973, **12**, 2781.



(355) X = S, Y = O
(356) X = O, Y = S

was obtained when the 5′-amino-5′-deoxyadenosine derivative (357) underwent ring closure under basic conditions (Scheme 130) stereoselectively in favour of the illustrated diastereoisomer (358); the isomer with the alternative configuration at the phosphorus atom was prepared by treatment of (358) with the *p*-nitrophenate ion.[655]

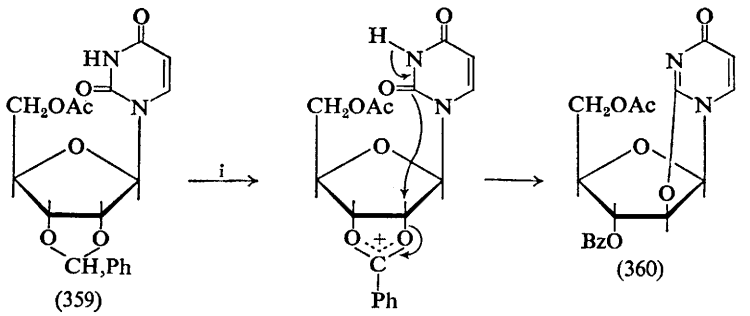


(357)

(358)

R^1 = *n*-octyl, R^2 = *p*-$NO_2C_6H_4$

Scheme 130



(359)

(360)

Reagent: i, Ph_3CBF_4

Scheme 131

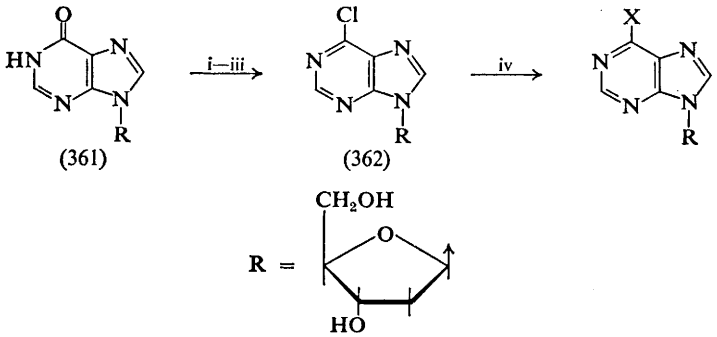
[655] A. Murayama, B. Jastorff, H. Hettler, and F. Cramer, *Chem. Ber.*, 1973, **106**, 3127.

Benzyl ether intermediates were employed in syntheses of 2′- and 3′-*O*-acyluridines, and migration of the 2′- and 3′-*O*-pivaloyl esters was investigated.[225] Both 2′,3′- and 3′,5′-cyclic carbonates of nucleosides have been described,[263] and a number of nucleoside 5′-carbamates have been synthesized by standard methods.[656] Trityl fluoroborate has been used to convert 2,3-benzylidene acetals into 2,3-benzoxonium ions, hydrolysis of which led to monobenzoates (see Scheme 28); however, the 2′,3′-*O*-benzylideneuridine (359) gave the 2,2′-cyclonucleoside (360) (see Scheme 131).[206]

Reagents for converting vicinal glycols into acylated halogenohydrin derivatives of nucleosides and some applications thereof are described in Chapter 7.

Reactions

Lithium derivatives of trimethylsilylated pyrimidine nucleosides have been used to introduce deuterium labelling (as in 6-[^{2}H]thymidine) and ^{14}C-labelled methyl groups into the molecules.[657] 2′-Deoxyinosine (361), obtained from 2′-deoxyadenosine by the action of adenosine deaminase, has been acylated and treated with thionyl chloride in DMF to give the related 6-chloropurine 2′-deoxynucleoside (362) after deacylation; the chloro-group of the latter compound was readily displaced by nucleophiles (Scheme 132).[658] The purinium salt (363) was obtained by quaternization



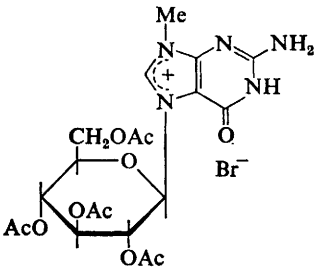
(361) (362)

Reagents: i, $(CF_3CO)_2O$; ii, $SOCl_2$–DMF; iii, MeOH–Al_2O_3; iv, HX = NH_2OH, $BnNH_2$, Me_2NH, MeSH, or H_2S

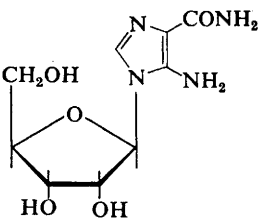
Scheme 132

of the free base in DMF,[659] and the purine ring of inosine underwent cleavage in aqueous alkali at elevated temperatures to give the imidazole nucleoside (364).[660] The 'ammonia adduct' previously obtained by the

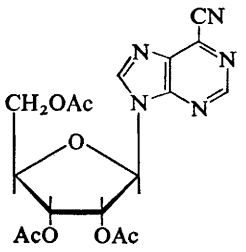
[656] W. C. Fleming, W. W. Lee, and D. W. Henry, *J. Medicin. Chem.*, 1973, **16**, 570.
[657] L. Pichat, J. Godbillon, and M. Herbert, *Bull. Soc. chim. France*, 1973, 2712, 2715.
[658] M. J. Robins and G. L. Basom, *Canad. J. Chem.*, 1973, **51**, 3161.
[659] K. Eistetter and W. Pfleiderer, *Chem. Ber.*, 1973, **106**, 1389.
[660] Y. Suzuki, *Chem. Letters*, 1973, 547 (*Chem. Abs.*, 1973, **79**, 53 726d).



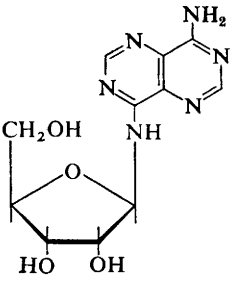
(363)



(364)

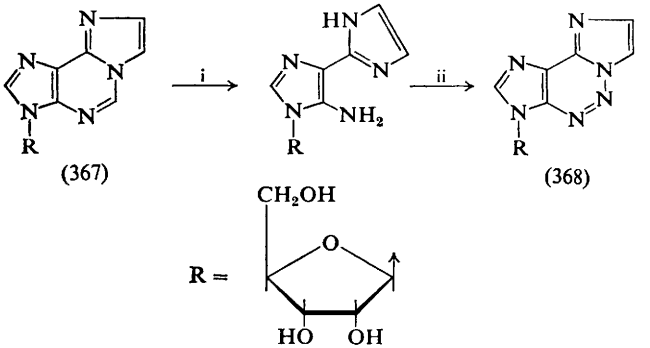


(365)



(366)

action of ammonia on 6-cyano-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (365) has been shown, by *X*-ray methods, to be 4-amino-8-(β-D-ribofuranosyl)aminopyrimidino[5,4-*d*]pyrimidine (366).[661] 1,N^6-Etheno-adenosine (367), a fluorescent nucleoside, was converted into a new fluorescent nucleoside, 2-aza-1,N^6-ethenoadenosine (368), on sequential treatment with base and nitrous acid (Scheme 133).[662]



(367) (368)

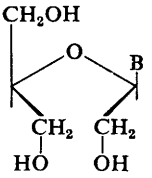
Reagents: i, NaOH; ii, HNO_2

Scheme 133

[661] H. M. Berman, *Tetrahedron Letters*, 1973, 3099.
[662] K. F. Yip and K. C. Tsou, *Tetrahedron Letters*, 1973, 3087.

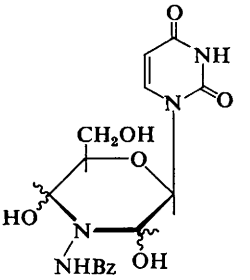
The short-lived radicals formed by photochemical reaction of nucleosides and hydrogen peroxide have been trapped as nitroxide radicals, and were shown to be formed by abstraction of hydrogen atoms from the sugar moieties by hydroxy-radicals.[663]

Sequential periodate oxidation and borohydride reduction have been used to prepare a number of triols (369) from nucleosides.[664] The adduct



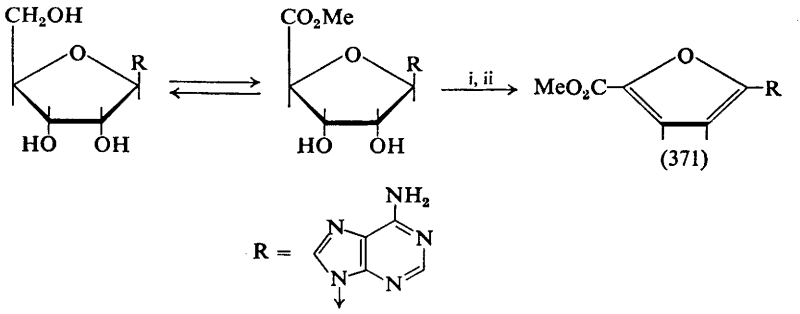
B = pyrimidine or purine

(369)



(370)

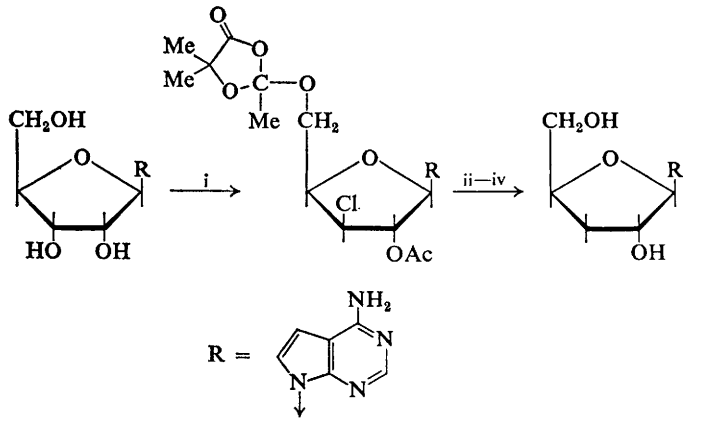
formed by reaction of periodate-oxidized uridine with benzoylhydrazine has now been shown to possess the morpholine structure (370).[665] An oxidation–reduction process has been adopted as an unusual method for protecting the 5′-hydroxymethyl group in the synthesis of nucleoside derivatives (Scheme 134); in the course of the work, the furanyl nucleoside (371) was obtained.[666] Details of 'deoxygenating' procedures applicable to nucleosides, and related to similar procedures described in Chapter 7,



Reagents: i, TsCl–py; ii, KOBu^t–DMF

Scheme 134

[663] C. Lagercrantz, *J. Amer. Chem. Soc.*, 1973, **95**, 220.
[664] R. R. Rossi and L. M. Lerner, *J. Medicin. Chem.*, 1973, **16**, 457.
[665] A. S. Jones and R. T. Walker, *Carbohydrate Res.*, 1973, **26**, 255.
[666] R. R. Schmidt, R. Machat, and U. Schloz, *Chem. Ber.*, 1973, **106**, 1256.

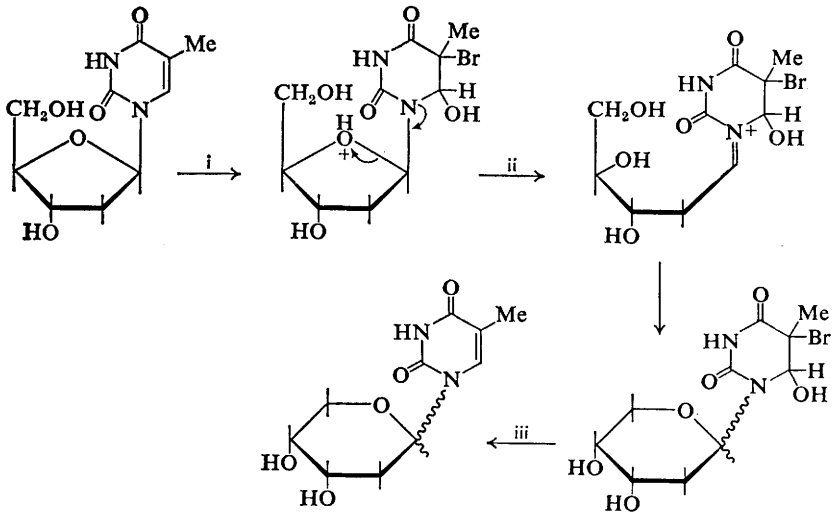


Reagents: i, $Me_2C(OAc)COCl$; ii, NaI–MeCN; iii, H_2–Pd; iv, NH_3–NeOH

Scheme 135

have been made available, and are illustrated with tubercidin in Scheme 135; a corresponding set of reactions on the *C*-nucleoside formycin gave 2′- and 3′-deoxy-derivatives in the ratio 2 : 3.[667]

Thymidine has been made to undergo anomerization and ring expansion by the temporary addition of hypobromous acid (Scheme 136).[668] It has



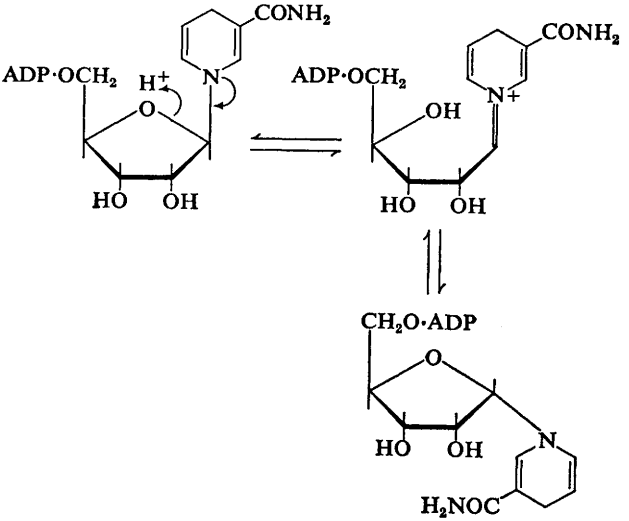
Reagents: i, Br_2–H_2O; ii, H^+; iii, Zn–HOAc

Scheme 136

[667] M. J. Robins, J. R. McCarthy, R. A. Jones, and R. Mengel, *Canad. J. Chem.*, 1973, **51**, 1313.

[668] J. Cadet and R. Teoule, *Carbohydrate Res.*, 1973, **29**, 345.

been suggested that the 10—15% of the α-anomer of nicotinamide adenine dinucleotide (NAD) previously found in commercial samples of NAD results from anomerization of NADH during the extraction procedure; it was demonstrated that α- and β-NADH equilibrate in either neutral or mildly alkaline conditions ($K\alpha/\beta = 0.18$), and a possible mechanism is shown in Scheme 137.[669] The glycosyl bond of 6-methylcytidine is

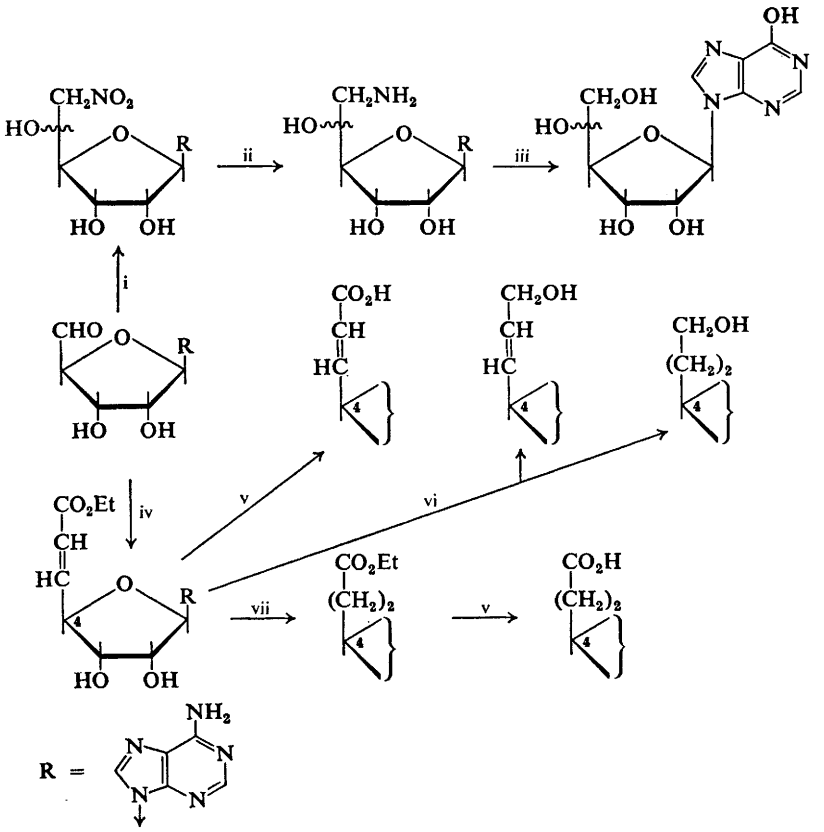


Scheme 137

hydrolysed in acidic media, and explanations based on hydrogen-bonding or steric effects were offered for the unexpected difference between this compound and cytidine, which is stable to acid.[670] Another unusual bond-cleavage in nucleosides has been reported; thus, it was found that adenosine, cytidine, uridine, and certain derivatives thereof released the free bases on treatment with potassium t-butoxide in anhydrous mixtures of t-butanol–*p*-dioxan.[671] The reactions require at least one of the D-ribosyl hydroxy-groups to be unsubstituted, and a number of possible explanations were advanced to account for the cleavage.

Reactions of the 5′-aldehydo-form of adenosine with nitromethane and with a Wittig reagent have been used to synthesize a series of adenosine analogues; these reactions are summarized in Scheme 138.[672] A number of analogues of *S*-adenosyl-L-homocysteine (and the related sulphoxides)

[669] E. L. Jacobson, M. K. Jacobson, and C. Bernofsky, *J. Biol. Chem.*, 1973, **248**, 7891.
[670] R. E. Notari, D. T. Witiak, J. L. Deyoung, and A. J. Lin, *J. Medicin. Chem.*, 1972, **15**, 1207.
[671] H. Follmann, *Tetrahedron Letters*, 1973, 397.
[672] T. E. Walker, H. Follmann, and H. P. C. Hogenkamp, *Carbohydrate Res.*, 1973, **27**, 225.



Reagents: i, CH_3NO_2; ii, H_2–Pt; iii, HNO_2; iv, $Ph_3P{=}CHCO_2Et$; v, NaOH; vi, $LiAlH_4$; vii, H_2–Pd

Scheme 138

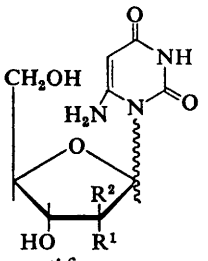
were obtained from suitably protected nucleoside 5′-toluene-*p*-sulphonates.[673]

Acetylations and reductions of keto-nucleosides are referred to in Chapters 16 and 22.

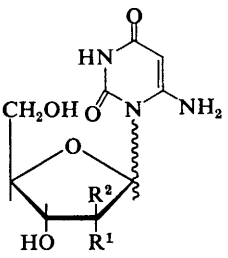
Physical Measurements

An interesting study has been carried out on the barriers to rotation about the glycosidic bond of the aminouridine (372) and 2-deoxy-α-D-*erythro*-pentofuranosyl (373) and α-D-arabinofuranosyl analogues (374) thereof; ΔG^* values of 9—13 kcal mol^{-1} were determined, and the *anti* form is

[673] V. N. Rekunova, I. P. Rudakova, and A. M. Yurkevich, *Tetrahedron Letters*, 1973, 2811.



anti-form



syn-form

(372) R^1 = OH, R^2 = H (β-anomer)
(373) R^1 = R^2 = H (α-anomer)
(374) R^1 = H, R^2 = OH (α-anomer)

thermodynamically preferred in each case.[674] Similar results were obtained with an isopterin nucleoside.[614] The *cis*- and *trans*-dichlorodiammino-platinums exhibit very different biological activities; the former is an active antitumour agent, whereas the latter is not. The interactions of these metal complexes with nucleosides have been studied by u.v. spectroscopy, and the positions of binding were located;[674a] the *cis*-isomer can bind mono- or bi-functionally, whereas the *trans*-isomer binds monofunctionally only. However, no binding was observed with some nucleosides. The complexing of Group II metal ions with nucleosides in DMSO solution has been studied by n.m.r. spectroscopy.[524]

Chapters 23 and 24 contain a number of references relating physical measurements on nucleosides, and papers describing c.d. studies on nucleosides are mentioned in Chapter 25.

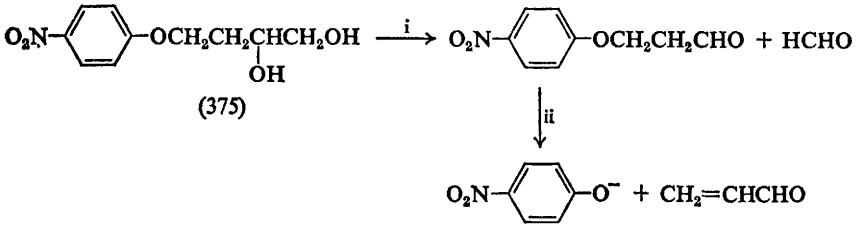
[674] J. C. Jochims, W. Pfleiderer, K. Kabayashi, G. Ritzmann, and W. Hutzenlaub, *Chem. Ber.*, 1973, **106**, 2975.
[674a] S. Mansy, B. Rosenberg, and A. J. Thomson, *J. Amer. Chem. Soc.*, 1973, **95**, 1633.

22
Oxidation and Reduction

Oxidation

Methods for oxidizing D-glucose to D-gluconic acid have been reviewed,[675] and the use of a platinum-catalysed oxidation in alkaline solution for effecting this conversion has been dealt with specifically in another report.[676]

A new spectrophotometric procedure for the determination of periodate ion has been described, and it is claimed to be advantageous for oxidations involving 10^{-6}—10^{-8} mole of a glycol.[677] The residual periodate ion is allowed to react with the glycol (375); the resulting aldehyde undergoes β-elimination on addition of a basic buffer to give the *p*-nitrophenate anion, which is measured spectrophotometrically at 400 nm (see Scheme 139). A warning has been given that spectrophotometric determination of



Reagents: i, IO_4^-; ii, HO^-

Scheme 139

the uptake of periodate in the oxidation of methyl 2-acetamido-2-deoxy-D-glucopyranosyl residues is unreliable, since the products of oxidation absorb at the wavelength of light (260 or 222.5 nm) used for the assay.[678] Initial cleavage in the periodate oxidation of methyl α-D-glucopyranoside has been shown to take place between C-3 and C-4, whereas it occurs between C-2 and C-3 in the 6-*O*-trityl derivative.[679]

[675] H. G. J. De Wilt, *Ind. and Eng. Chem.* (*Product Res. and Development*), 1972, **11**, 370 (*Chem. Abs.*, 1973, **78**, 43 862i).

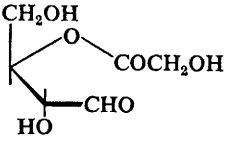
[676] H. G. J. De Wilt and H. S. Van Der Baan, *Ind. and Eng. Chem.* (*Product Res. and Development*), 1972, **11**, 374 (*Chem. Abs.*, 1973, **78**, 43 873q).

[677] D. H. Rammler, R. Bilton, R. Haugland, and C. Parkinson, *Analyt. Biochem.*, 1973, **52**, 198.

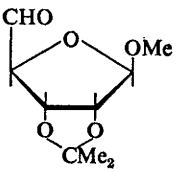
[678] J. Elting, C.-C. Huang, and R. Montgomery, *Carbohydrate Res.*, 1973, **28**, 387.

[679] R. G. Krylova, S. N. Ryadovskaya, L. I. Kostelian, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2068.

Following earlier reports on the application of silver carbonate on Celite as an oxidizing reagent in carbohydrate chemistry (see Vol. 6, p. 8), Morgenlie has described the reaction of this reagent with 2-ketoses in general.[11] D-Fructose, for example, afforded the D-erythrose ester (376) initially, and other 2-ketoses were also oxidized at the 2,3-diol grouping. In this respect, the reagent is functionally similar to lead tetra-acetate and the periodate ion. Although not yet used with carbohydrates, activated manganese dioxide in dichloromethane has been shown to cleave *vic*-glycols with the formation of carbonyl compounds; however, a conformationally rigid diol, with a dihedral angle of 180° subtended by the hydroxy-groups, resisted oxidation.[680]



(376)



(377)

The chromium trioxide–bipyridyl complex has been demonstrated to be an efficient reagent for the oxidation of carbohydrate primary alcohols to aldehydes, and methyl carbinols to methyl ketones.[477] It is claimed to be superior to either the Pfitzner–Moffatt reagent (DMSO–DCC) or the photolysis of azides for the preparation of aldehydes; for example, the aldehyde (377) was obtained in 75% yield from methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside. Unfortunately, such endocyclic, secondary alcohol derivatives as 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and 1,6-anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose were essentially inert toward the reagent, although a protected hemiacetal was oxidized to the corresponding 1,4-lactone in high yield.

The oxidation of ketoses by alkaline hydrogen peroxide is mentioned in Chapter 2.

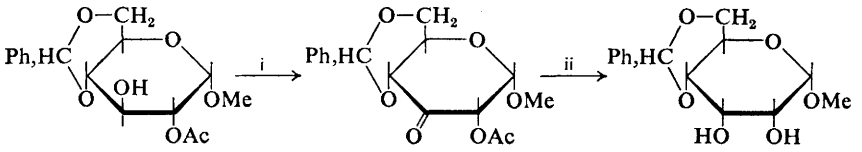
Reduction

The hydrogenation of D-glucose in the presence of nickel catalysts containing other metals such as aluminium, rhenium, or tin has been discussed at length by Russian authors.[681–683]

The stereochemistries which result from the reductions of isopropyl 3,4,6-tri-*O*-acetyl-α-D-*arabino*- and -*lyxo*-pyranosid-2-uloses [(19) and (20),

[680] G. Ohloff and W. Giersch, *Angew. Chem. Internat. Edn.*, 1973, **12**, 401.
[681] O. S. Popov, D. V. Sokol'skii, and E. M. Sul'man, *Doklady Akad. Nauk S.S.S.R.*, 1972, **205**, 1156 (*Chem. Abs.*, 1973, **78**, 4438g).
[682] N. K. Nadirov, D. V. Sokol'skii, Sh. Kh. Khandodzhaev, and A. M. Ashirov, *Zhur. priklad. Khim.*, 1972, **45**, 2652.
[683] F. B. Bizhanov, D. V. Sokol'skii, and U. I. Yunusov, *Izvest. Akad. Nauk Kazakh. S.S.R., Ser. khim.*, 1972, **22**, 80.

respectively] by metal hydrides and by catalytic methods have been compared, and the results are given in Table 1 (see p. 20).[89] Other papers have discussed the reduction of carbonyl compounds with metal hydrides, placing particular emphasis on the stereoselectivity achieved. In this context, two new reducing agents, potassium tri-isopropoxyborohydride [684] and tri-s-butylborohydride,[684a] possessing a remarkably high degree of stereoselectivity have been reported. Although not yet employed with carbohydrate systems, they may well prove useful. A convenient synthesis of methyl 4,6-*O*-benzylidene-α-D-allopyranoside (378), using an oxidation–reduction sequence, is shown in Scheme 140; application of the same

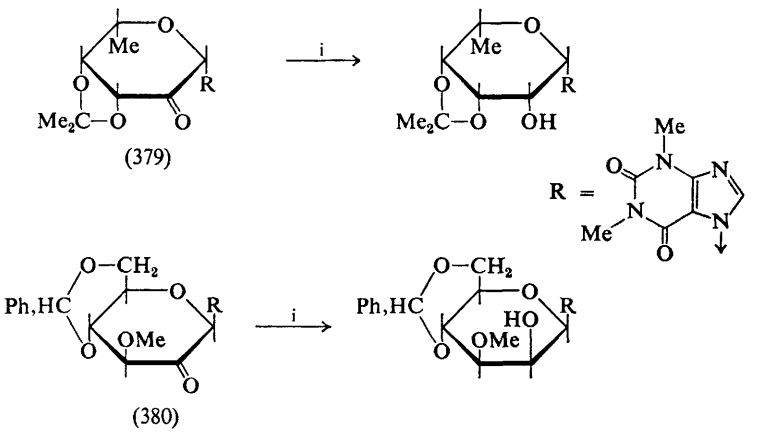


(378)

Reagents: i, Ac_2O–DMSO; ii, $NaBH_4$–DMF–MeOH

Scheme 140

sequence to the β-glycoside gave equal amounts of the *gluco-* and *allo-*isomers.[685] Borohydride reductions of the 2′-keto-nucleosides (379) and (380) were found to be stereospecific (Scheme 141), with attack taking place from the less-hindered side of the molecule in each case.[686]



(379)

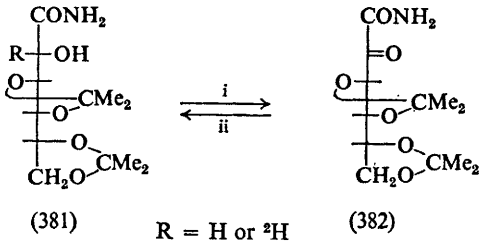
(380)

Reagent: i, $NaBH_4$

Scheme 141

[684] C. A. Brown, S. Krishnamurthy, and S. C. Kim, *J.C.S. Chem. Comm.*, 1973, 391.
[684a] C. A. Brown, *J. Amer. Chem. Soc.*, 1973, **95**, 4100.
[685] Y. Kondo, *Carbohydrate Res.*, 1973, **30**, 386.
[686] K. Antonakis, M.-J. Arvor-Egron, and F. Leclercq, *Carbohydrate Res.*, 1972, **25**, 518.

Two papers have reported the reductions of exocyclic carbonyl groups. Reduction of the hexulosonamide (382) gave predominantly the D-gluconamide derivative (381) (Scheme 142), which had originally been oxidized

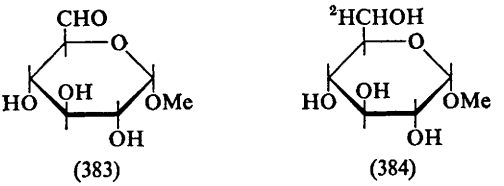


(381) R = H or ^2H (382)

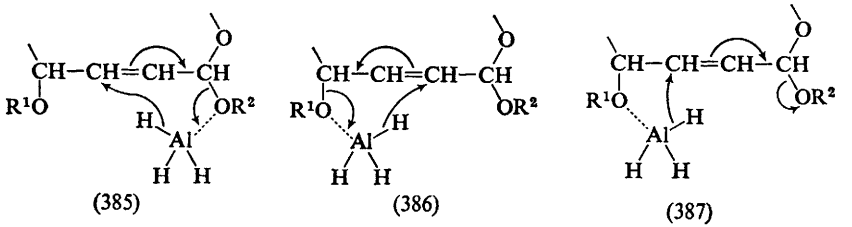
Reagents: i, Ac_2O–DMSO; ii, $NaBH_4$ or NaB^2H_4

Scheme 142

to produce the ketone; reduction with sodium borodeuteride was used to introduce deuterium at C-2 of the D-gluconamide.[687] Borodeuteride reduction of the dialdoside (383) gave a 3 : 2 mixture of the 6(*S*)- and 6(*R*)-forms of methyl 6-deuterio-α-D-glucopyranoside (384).[688] The deuterium-decoupled n.m.r. spectra of such derivatives as methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-6-deuterio-α-D-glucopyranoside permitted the configurations at C-6 to be assigned.



(383) (384)

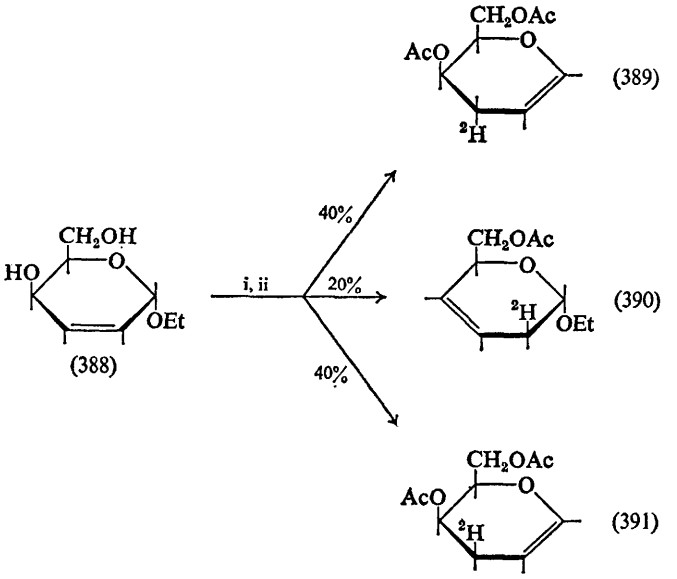


(385) (386) (387)

Tam and Fraser-Reid have examined the substrate geometry and the reaction specificity of reductive rearrangements of unsaturated acetals to vinyl ethers brought about by chloride-free lithium aluminium hydride in

[687]D. Gagnaire and F. R. Taravel, *Carbohydrate Res.*, 1973, **27**, 239.
[688]D. Gagnaire, D. Horton, and F. R. Taravel, *Carbohydrate Res.*, 1973, **27**, 363.

ethers.[689] Three types of transition state [(385)—(387)] were proposed. Reduction of the unsaturated hexopyranoside (388) with lithium aluminium deuteride afforded (389), (390), and (391), indicating that all three modes of reaction are operating (Scheme 143).



Reagents: i, $LiAl^2H_4$; ii, Ac_2O–py

Scheme 143

[689] S. Y.-K. Tam and B. Fraser-Reid, *Tetrahedron Letters*, 1973, 4897.

23

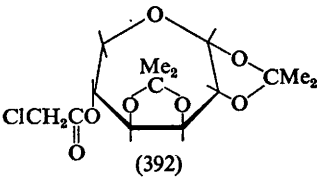
N.M.R. Spectroscopy and Conformational Features of Carbohydrates

Tentative rules for the conformation nomenclature of five- and six-membered rings in monosaccharides have been published and await approval by the International Union of Pure and Applied Chemistry (IUPAC).[690]

Reviews have appeared on the conformational analysis, by n.m.r. spectroscopy, of monosaccharides and polysaccharides (in Russian)[691] and of various sugar acetates (in Japanese), with particular emphasis on the configuration of the C-1 linkages.[692] ^{1}H N.m.r. spectra have been reported and discussed for the following groups of compounds: acetylated steroid and triterpenoid glycosides,[119, 693] acetylated galactocerebrosides,[694] and methyl 3,6-anhydro-α-D-galactopyranoside, 3,6-anhydro-D-galactose dimethyl acetal, and the corresponding di- and tri-acetates.[695]

The conformation of the planar imido-ring relative to the pyranoid ring in acetylated *N*-phthalimido-D-glucopyranoses has been studied.[696]

The crystal and molecular structures of 5-*O*-chloroacetyl-1,2:3,4-di-*O*-isopropylidene-α-D-glucoseptanose (392) have been determined, and the seven-membered ring has been shown to adopt a $^{5,6}C_2$ twist-chair conformation.[697]



(392)

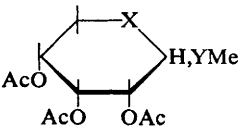
[690] J. C. P. Schwarz, *J.C.S. Chem. Comm.*, 1973, 505; *J.C.S. Perkin I*, 1973, 1912 (see pages following).
[691] V. P. Panov and R. G. Zhbankov, *Zhur. priklad. Spektroskopii*, 1973, **18**, 1103.
[692] M. Matsui, *Kagaku No Ryoiki*, 1973, **27**, 423 (*Chem. Abs.*, 1973, **79**, 32 192m).
[693] A. K. Dzizenko, V. V. Isakov, N. I. Uvarova, G. I. Oshitok, and G. B. Elyakov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 1351 (*Chem. Abs.*, 1973, **78**, 111 686h).
[694] M. Martin-Lomas and D. Chapman, *Chem. and Phys. Lipids*, 1973, **10**, 152.
[695] K. Izumi, *Carbohydrate Res.*, 1973, **27**, 278.
[696] M. Iwakawa and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1973, **46**, 1525.
[697] J. Jackobs, M. A. Reno, and M. Sundaralingam, *Carbohydrate Res.*, 1973, **28**, 75.

Two groups have used $J_{^{31}P,^{1}H}$ values to ascertain the structures of such 3-*C*-(dimethoxy)phosphinyl derivatives as (282) and (283),[514, 515] and mention has already been made of a hydration model for monosaccharides based on ^{17}O n.m.r. studies.[30]

Pyranoid Systems

Theoretical values of the energy barriers for $^{1}C_4 \rightleftarrows {}^{4}C_1$ interconversions have been derived, and agree well with values determined experimentally.[698] Free-energy calculations performed on aldohexopyranose penta-acetates were also in agreement with results obtained from n.m.r. studies.[699] The latter study has furnished information on the anomeric effect of an acetoxy-group, the effects of substituents on the conformation of the pyranoid ring, and the possible deviations in bond angles in different steric arrangements of side-groups.

Conformational equilibria for the triacetates of methyl α- and β-D-ribopyranosides (393), and the 1-thio (394), 5-thio (395), and 1,5-dithio



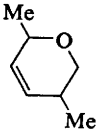
(393) X = Y = O
(394) X = O, Y = S
(395) X = S, Y = O
(396) X = Y = S

(396) analogues, have been examined by ^{1}H n.m.r. spectroscopy.[700] It appears that only methyl 2,3,4-tri-*O*-acetyl-β-D-ribopyranoside (β-393) exhibits a preference for the $^{1}C_4$ conformation in solution; the preference of the other glycosides for the $^{4}C_1$ conformation was rationalized in terms of anomeric, steric, and 'hockey-stick' effects. Similar results were obtained for the deacetylated glycosides in pyridine solution, although somewhat different results were obtained in the crystalline state as a consequence of intramolecular hydrogen bonding (see Chapter 24).

The chemical shifts of the anomeric protons for a series of perbenzoylated aldohexopyranoses and disaccharides have been reported, and the chemical shifts of H-1 were discussed in terms of the shielding effects exerted by the benzoyloxy-groups.[701] ^{1}H N.m.r. spectroscopic data have been obtained for eight isomeric methyl 2-amino-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-D-hexopyranosides, and the ^{13}C chemical shifts were compared with those of related compounds.[702] 220 MHz ^{1}H n.m.r. spectroscopy was used to

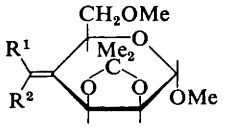
[698] A. A. Lugovoskoy, V. G. Dashevsky, and A. I. Kitaigorodsky, *Tetrahedron*, 1973, **29**, 287.
[699] K. S. Vijayalakshmi and V. S. R. Rao, *Carbohydrate Res.*, 1973, **29**, 427.
[700] N. A. Hughes, *Carbohydrate Res.*, 1973, **27**, 97.
[701] J. O. Deferrari, I. M. E. Thiel, and R. A. Cadenas, *Carbohydrate Res.*, 1973, **26**, 244.
[702] N. Gurudata and F. J. M. Rajabalee, *Canad. J. Chem.*, 1973, **51**, 1797.

determine the conformations of the ring systems of the four crystalline di-D-fructose dianhydrides[703] and to show that atractyloside is a β-D-glucopyranoside.[704] ^{1}H N.m.r. spectroscopic analysis of the conformations of the 2,5-dimethyl-5,6-dihydro-α-pyrans (397) should prove useful for comparison with those of 2- and 3-enopyranosides.[438]



(397)

Four papers have presented evidence of skew conformations for pyranoid rings bearing a *cis*-fused dioxolan ring. The presence of a 2,3-*O*-isopropylidene group on either methyl α-D-mannopyranoside or -talopyranoside causes the normally preferred $^4C_1$ conformation in solution to be slightly flattened, while the further addition of an *endo* 4-deoxy-4-*C*-methyl substituent to the latter compound results in the molecule adopting a $^3S_1$ skew conformation.[705] With an sp^2 carbon atom at position 4, as in monosubstituted derivatives of methyl 4-deoxy-2,3-*O*-isopropylidene-6-*O*-methyl-4-*C*-methylene-α-D-*lyxo*-hexopyranoside (398), a conformation



(398) R^1 = CN, R^2 = H
R^1 = H, R^2 = CN
R^1 = SMe, R^2 = H
R^1 = H, R^2 = SMe

close to $^3S_1$ is adopted, regardless of the configuration at the double bond. The $^0S_2$ skew conformation was indicated, by n.m.r. studies, for the 4,6-di- and 3,4,6-tri-*O*-methyl ethers of 1,2-*O*-isopropylidene-α-D-glucopyranose.[706] An *X*-ray crystallographic study of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-*exo*-ethoxyethylidene)-α-D-glucopyranose has demonstrated that the pyranoid ring assumes an approximately $^3S_5$ skew conformation, although there is some flattening of the ring in the vicinity of C-1 and C-2.[707] Similar examination of methyl 2,6-dichloro-2,6-dideoxy-3,4-*O*-isopropylidene-α-D-altropyranoside has revealed an unusual skew-boat conformation for the

[703] R. W. Binkley, W. W. Binkley, and A. A. Grey, *Carbohydrate Res.*, 1973, **28**, 365.
[704] G. Defaye, D. Horton, and J. D. Wander, *Bull. Soc. chim. France*, 1973, 615.
[705] J. M. J. Tronchet, F. Barbalat-Rey, and J. M. Chalet, *Carbohydrate Res.*, 1973, **30**, 229.
[706] C. Peciar, J. Alfoldi, R. Palovcik, and P. Kováč, *Chem. Zvesti*, 1973, **27**, 9 (*Chem. Abs.*, 1973, **78**, 160 003s).
[707] J. A. Heitmann and G. F. Richards, *Carbohydrate Res.*, 1973, **28**, 180.

pyranoid ring, with C-2 and C-5 being maximally displaced from the mean plane through the other ring atoms.[708]

Furanoid Systems

Most of the papers in this section refer to nucleosides and nucleotides, with interest divided between the conformations of the furanoid rings and those about the C-4′–C-5′ and C-1′–N bonds.

An extensive paper has appeared on the conformations of the sugar ring of D-ribofuranosyl nucleotides and nucleosides, using a detailed analysis of the proton–proton coupling constants.[709] Two twist forms, with C-2′ and C-3′ as the out-of-plane atoms, were found to represent energy minima, and the proportion of each form was determined from the observed $J_{1',2'}$ values. β-D-Ribofuranosylpurines were indicated to prefer the $^2T_3$ conformation, whereas pyrimidine analogues favour the $^3T_2$ conformation. The energetics of these systems were described in detail, and the work was extended to include mono- and di-nucleotides; deoxyribonucleosides and the 5′-phosphates favour the $^2T_3$ form. Variable-temperature, 220 MHz n.m.r. studies on 2′-deoxynucleosides have also indicated the favoured conformations of the sugar moiety of these molecules to be $^2V \rightleftarrows {}^2T_3 \rightleftarrows V_3$ and an alternative combination of $^0V \rightleftarrows {}^0T_4 \rightleftarrows V_4$.[710] 220 MHz n.m.r. studies have further revealed that methylation at O-2′ has little effect on the conformations adopted by uridine and cytidine in aqueous solution.[711] The variable-temperature, 100 MHz ^{1}H n.m.r. spectra of a number of derivatives of pyrimidine nucleotides have been measured in a range of solvents.[712] Both ^{1}H and ^{13}C n.m.r. studies of pyrimidine nucleosides in water have shown that most of the molecules preferentially adopt the *anti* conformation about the sugar–base linkage, and no significant changes were encountered either on changing the solvent to DMSO or on forming the 2′,3′-*O*-isopropylidene derivatives.[713] A related study (using ^{1}H n.m.r. spectroscopy) has also shown that the sugar–base torsion angles of uridine, 5,6-dihydrouridine, and β-*pseudo*-uridine are unaffected by a change of solvent from deuterium oxide to [$^2H_6$]DMSO, although the rotameric states about the C-4′–C-5′ bond are solvent-dependent.[714] Extended Hückel calculations have been used to predict the preferred conformations of adenosine 5′-phosphate,[715] while the conformations of uridine and its 5′-phosphate, and of the corresponding 6-azauracil analogues, have been

[708] G. H.-Y. Lin, M. Sundaralingam, and J. Jackobs, *Carbohydrate Res.*, 1973, **29**, 439.
[709] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333.
[710] K. N. Slessor and A. S. Stacey, *Carbohydrate Res.*, 1973, **27**, 407.
[711] F. E. Hruska, A. Mak, H. Singh, and D. Shugar, *Canad. J. Chem.*, 1973, **51**, 1099.
[712] D. K. Lavallee and C. L. Coulter, *J. Amer. Chem. Soc.*, 1973, **95**, 576.
[713] M. P. Schweizer, E. B. Banta, J. T. Witkowski, and R. K. Robins, *J. Amer. Chem. Soc.*, 1973, **95**, 3770.
[714] R. Deslauriers and I. C. P. Smith, *Canad. J. Chem.*, 1973, **51**, 833.
[715] D. Vasilescu, J. N. Lespinasse, F. Camous, and R. Cornillon, *F.E.B.S. Letters*, 1972, **27**, 335.

investigated by ^1H n.m.r. spectroscopy; in azauracil 5′-phosphate, gauche–gauche rotamers about the C-4′—C-5′ and C-5′—O-5′ bonds are apparently destabilized by a repulsive, electrostatic interaction between the 6-aza nitrogen and the negatively charged phosphate group.[716] $^4J_{^{31}P,^1H}$ Couplings have also been used to gain information on the preferred rotameric states about the C-4′–C-5′–O-5′ portion of nucleoside 5′-phosphates.[717]

Three papers from Smith's group have described work on cyclic phosphates. ^1H N.m.r. spectral analyses for adenosine and thymidine 3′,5′-cyclic phosphates showed that both furanoid and phosphate rings are rigid, with the phosphate rings assuming chair conformations [718]—these findings accord with those of *X*-ray crystallography; $J_{^{31}P,^1H}$ values were used to establish a relation between the coupling constants and dihedral angles that could be applied to acyclic nucleotides and oligonucleotides. ^{13}C N.m.r. studies on six nucleoside 3′,5′-cyclic phosphates confirmed the above finding, and the use of $J_{^{13}C,^{31}P}$ values as probes for the molecular conformation was discussed.[719] Related ^1H and ^{13}C n.m.r. studies on four nucleoside 2′,3′-cyclic phosphates showed that these molecules, unlike the 3′,5′-cyclic phosphates, are in rapid conformational equilibrium.[720]

Di-, Oligo-, and Poly-saccharides

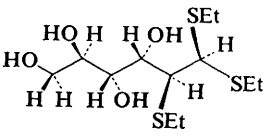
The chemical shifts in the ^1H n.m.r. spectra of various 1 → 1-, 1 → 2-, 1 → 3-, 1 → 4-, and 1 → 6-linked disaccharide octa-acetates have been correlated with the configuration at the anomeric centre.[721]

Disaccharides and the mutarotation thereof have been studied by ^{13}C n.m.r. spectroscopy.[722] Detailed analyses have been reported of the ^{13}C n.m.r. spectra of D-gluco-bioses and -trioses [723] and D-manno-bioses and -trioses [724] in anticipation that the assignments will aid in structural studies on glucans and mannans, respectively. Other pertinent examples are dealt with in a later section on ^{13}C n.m.r. spectroscopy.

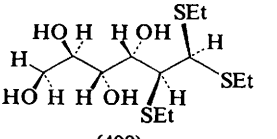
Acyclic Derivatives

The conformations of 2-*S*-ethyl-2-thio-D-mannose diethyl dithioacetal have been determined both in solution (by ^1H n.m.r. spectroscopy) and

[716] D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *Canad. J. Chem.*, 1973, **51**, 2571.
[717] R. H. Sarma, R. J. Mynott, D. J. Wood, and F. E. Hruska, *J. Amer. Chem. Soc.*, 1973, **95**, 6457.
[718] B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1973, **95**, 2873.
[719] R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1973, **95**, 2878.
[720] R. D. Lapper and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1973, **95**, 2880.
[721] V. V. Isakov, A. K. Dzizenko, V. I. Govorchenko, V. A. Denisenko, and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, **8**, 425.
[722] W. Voelter, V. Bilik, and E. Breitmaier, *Coll. Czech. Chem. Comm.*, 1973, **38**, 2054.
[723] T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J.C.S. Perkin I*, 1973, 2425.
[724] P. A. J. Gorin, *Canad. J. Chem.*, 1973, **51**, 2375.



(399)



(400)

in the crystalline state (by *X*-ray diffraction).[725] Two planar, extended, zigzag conformations [(399) and (400)], differing only in the rotameric states about the C-5–C-6 bond, were detected in solution, whereas two slightly different conformations, approximating to (400), were found in the crystal. Similar results were obtained for the conformations of a number of 2-(polyhydroxyalkyl)- and 2-(polyacetoxyalkyl)-benzothiazoles in DMSO and chloroform solutions; compounds possessing the D-*arabino*, D-*galacto*, and D-*manno* configurations were shown to adopt an extended, planar conformation in both series, whereas those having D-*xylo*, D-*ribo*, and D-*gluco* configurations prefer a bent conformation in order to relieve 1,3-dipolar interactions.[726] The ^{1}H n.m.r. spectra of a series of alditol acetates have been recorded.[726a]

Lanthanide Shift Reagents

Angyal has shown that lanthanide ions can be used with aqueous solutions of carbohydrates to produce significant shifts of the proton signals, provided that the system possesses a suitable steric arrangement, *viz.* an *ax.*, *eq.*, *ax.* sequence of oxygen atoms on a six-membered ring (as, for example, in methyl 2-*C*-hydroxymethyl-β-D-ribopyranoside), for complexing.[727] The unidentate complex formed between 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and a lanthanide shift reagent has been shown (by n.m.r. methods) to be rigid at the point of attachment, signifying that the configuration of the complex can be ascertained with confidence.[728]

The stereochemistry at the tertiary centre of derivatives of five branched-chain sugars has been determined with the aid of the paramagnetic lanthanide complex tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium(III).[729]

^{13}C N.M.R. Spectroscopy

Two papers have reported work of potential value with regard to structural investigations of oligo- and poly-saccharides. Both the ^{1}H and ^{13}C n.m.r.

[725] A. Ducruix, C. Pascard-Billy, D. Horton, and J. D. Wander, *Carbohydrate Res.*, 1973, **29**, 276.
[726] L. Szilágy, R. Bognár, and I. Farkas, *Carbohydrate Res.*, 1973, **26**, 305.
[726a] L. Maijs, L. S. Bresler, I. Yu. Tsereteli, E. I. Karabanova, and B. Pavare, *Latv. P.S.R. Zinat. Akad. Vestis, Khim. Ser.*, 1972, 469 (*Chem. Abs.*, 1973, **78**, 4452g).
[727] S. J. Angyal, *Carbohydrate Res.*, 1973, **26**, 271.
[728] I. M. Armitage, L. D .Hall, A. G. Marshall, and L. G. Werbelow, *J. Amer. Chem. Soc.*, 1973, **95**, 1437.
[729] S. D. Gero, D. Horton, A. M. Sepulchre, and J. D. Wander, *Tetrahedron*, 1973, **29**, 2963.

spectra of methyl 2,3,4,6-tetra-*O*-methyl-α- and -β-D-glucopyranosides have been completely assigned by reference to derivatives containing either CD_3O or $^{13}CH_3O$ groups and by the use of heteronuclear, off-resonance spin-decoupling.[730] Partially methylated D-glucosides, obtained (for example) on methanolysis of a permethylated disaccharide, could be identified after further methylation with labelled methyl groups and comparison of the ^1H or ^{13}C n.m.r. spectra with those of the reference compounds. The ^{13}C n.m.r. spectra of the 2-acetamido-2-deoxy-D-hexoses and some of their 3-*O*-acetyl and 1-phosphate derivatives have been assigned and used in studies of polysaccharide antigens.[340]

Other compounds for which ^{13}C n.m.r. spectra have been reported and, in some cases, assigned include the isomeric methyl 2-amino-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-D-hexopyranosides,[702] D-glucose 3- and 6-sulphates,[731] a number of 9-(pento-furanosyl and -pyranosyl)adenines,[732] flavin adenine dinucleotide,[733] and showdomycin and β-*pseudo*-uridine.[734] The ^{13}C resonances of formycins A and B and related nucleosides exhibited significant line broadening as a consequence of prototropic tautomerism in the base moieties.[567, 735] The ^{13}C n.m.r. spectra of the amino-glycoside antibiotics gentamicin [559] and butirosin A [736] have been assigned, and should prove helpful in the elucidation of related structures; the effects, on the spectrum, of protonation and *N*-trifluoroacetylation of butirosin A were also studied.

Geminal ^{13}C–^1H couplings at C-1 of monosaccharide derivatives have been related to the anomeric configuration; an equatorial proton at C-1 generally exhibits a coupling constant approximately 10 Hz larger than its axial counterpart.[737] The anomeric compositions of equilibrated solutions of D-fructofuranose 6-phosphate and the corresponding 1,6-diphosphate have been estimated by ^{13}C n.m.r. methods.[244]

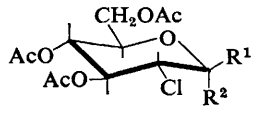
Two changes in the ^{13}C n.m.r. spectra of polyhydroxy-compounds have been observed on formation of complexes with borate. In one type of change, the chemical shift remained constant, but broadening of the signals of ^{13}C atoms in the vicinity of the complex occurred; whereas, in the other type, the signal remained sharp, but changes in chemical shift took place.[738] The results were used to investigate the structures of different types of borate complex and to determine the proportion of complex to starting material. In a related study of the complexing of sugars with

[730] J. Havercamp, J. P. C. M. van Dongen, and J. F. G. Vliegenthart, *Tetrahedron*, 1973, **29**, 3431.
[731] S. Honda, H. Yuki, and K. Takiura, *Carbohydrate Res.*, 1973, **28**, 150.
[732] E. Breitmaier and W. Voelter, *Tetrahedron*, 1973, **29**, 227.
[733] E. Breitmaier and W. Voelter, *European J. Biochem.*, 1972, **31**, 234.
[734] M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocyclic Chem.*, 1973, **10**, 427.
[735] M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Heterocyclic Chem.*, 1973, **10**, 431.
[736] P. W. K. Woo and R. D. Westland, *Carbohydrate Res.*, 1973, **31**, 27.
[737] K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Letters*, 1973, 1037.
[738] P. A. J. Gorin and M. Mazurek, *Carbohydrate Res.*, 1973, **27**, 325.

sodium diphenylborinate, certain of the ^{13}C n.m.r. spectra exhibited sharp singlets for the borinate complex, implying that ^{13}C–^{11}B coupling does not occur.[739] The interactions of borate with sugars in aqueous solution have also been examined by ^{11}B n.m.r. spectroscopy.[260] Chemical-shift values were used to establish the size of the boron-containing ring; thus, D-glucose was indicated to form 1,2-furanoid and 1,2-pyranoid complexes in the presence of borax and benzeneboronic acid.

Longitudinal Relaxation Times

Useful information can be derived from longitudinal nuclear relaxation times (T_1 values), and Hall's group has continued their interesting studies in this area, where, fortunately, all the proton relaxation times are much shorter than those of water, thereby enabling studies to be carried out in aqueous solutions. Characteristic differences in T_1 values exist for protons having axial or equatorial dispositions with respect to the ring system; two relaxation pathways, involving either 1,3,5-triaxial or vicinal-gauche interactions, are available to axial protons, whereas only the vicinal-gauche interaction can operate for equatorial protons.[740, 741] Thus, H-1 and H-3 of the β-anomer of compound (402) have shorter relaxation times than the



(401) R^1 = H, R^2 = OBz
(402) R^1 = OBz, R^2 = H

	τ_1 values/s (401)	(402)
H-1	4.3	1.8
H-2	2.1	3.3
H-3	3.5	2.5
H-4	2.8	2.8
H-5	1.3	1.1
H-6,6′	0.87	0.76

corresponding protons in the α-anomer (401), whereas the reverse holds for H-2. Advantage can be taken of the shorter relaxation times of H-5 and H-6,6′ to simplify the spectra; for example, increasing the delay time between the initial and monitoring pulses effectively removes the signals due to H-5 and H-6,6′ from the spectra. The T_1 values of anomeric protons of oligosaccharides have also been measured; as with monosaccharides, the axially oriented proton at the reducing end-group always has a shorter relaxation time than its equatorial counterpart.[742] Moreover, the anomeric-proton resonance of the non-reducing end-group also shows a configurational dependence, and T_1 values of these protons are much shorter than those of H-1 at the reducing end, so providing a potentially useful basis for assigning the individual resonances of the anomeric protons of oligosaccharides.

[739] P. A. J. Gorin and M. Mazurek, *Canad. J. Chem.*, 1973, **51**, 3277.
[740] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1973, **27**, 286.
[741] C. W. M. Grant, L. D. Hall, and C. M. Preston, *J. Amer. Chem. Soc.*, 1973, **95**, 7742.
[742] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1973, **29**, 522.

Nuclear relaxation times have also been used to study the binding of methyl α- and β-D-glucopyranosides (uniformly labelled with 14% ^{13}C) to Zn^{2+}- and Mn^{2+}-forms of concanavalin A.[743] The T_1 values for the ring carbons were uniformly shortened when a glycoside was bound to the Zn^{2+}-form, whereas they were selectively shortened when a glycoside was bound to the Mn^{2+}-form. The information so obtained enabled the three-dimensional orientation of each glycoside relative to the sites of binding of Mn^{2+} ions in concanavalin A to be determined. Each glycoside was indicated to remain in the $^4C_1$ conformation with the hydroxymethyl group, at a distance of 10 Å, nearest to the Mn^{2+} ion.

[743] C. F. Brewer, H. Sternlicht, D. M. Marcus, and A. P. Grollman, *Biochemistry*, 1973, **12**, 4448.

24
Other Physical Methods

I.R. Spectroscopy

As in previous years, only a few papers relating specifically to i.r. spectroscopy have been published, but the technique has continued to be widely used on a routine basis.

Far-i.r. measurements on D-glucose, D-sorbose, sucrose, and cellobiose at liquid-helium temperatures have shown that absorption bands are much stronger at low temperatures.[744] Hydrogen bonding between α-D-glucose and various solvents has been studied by near-i.r. spectroscopy, and various thermodynamic parameters of hydrogen bonding have been obtained.[745] Related investigations on several crystalline glycosides,[746] and on (β-aryloxyethyl) β-D-glucosides and 1,2-*O*-ethylene-β-D-glucopyranose[747] were also reported.

Raman spectra of a number of carbohydrates showed scattering at *ca.* 3370 cm^{-1} that could be related to the O–H bond, and it was observed that the 2850–3000 cm^{-1} region is useful for analytical purposes.[748]

Mass Spectrometry

Mass spectrometry is now used widely for the analysis of carbohydrates, and a useful review on the applications of mass spectrometry to carbohydrate chemistry has appeared.[749]

Among monosaccharides examined by mass spectrometry were 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (as well as deuteriated derivatives),[750] 3,6-dideoxy-α-L-*xylo*-hexopyranosides of long-chain, aliphatic alcohols (ascarosides),[751] methyl 4,6-*O*-benzylideneglycosides and

[744] M. Hineno and H. Yoshinaga, *Spectrochim. Acta*, 1972, **28A**, 2263.
[745] G. F. Trott, E. E. Woodside, K. G. Taylor, and J. C. Deck, *Carbohydrate Res.*, 1973, **27**, 415.
[746] G. A. Kogan, V. M. Tul'chinsky, M. L. Schulman, S. E. Zurabyan, and A. Y. Khorlin, *Carbohydrate Res.*, 1973, **26**, 191.
[747] A. Mesquida, *Rev. Acad. Cienc. Exact., Fis. Quim. Nat. Zaragoza*, 1972, **27**, 121 (*Chem. Abs.*, 1972, **77**, 152 504v).
[748] I.-C. Wang and C. H. Ting, *J. Chinese Chem. Soc.* (*Formosa*), 1972, **19**, 63.
[749] 'Biochemical Applications of Mass Spectrometry', ed. G. R. Waller, Wiley–Interscience, New York, 1972.
[750] D. Horton, E. Just, and J. D. Wander, *Org. Mass. Spectrometry*, 1972, **6**, 1121.
[751] G. E. Tarr and H. K. Schnoes, *Arch. Biochem. Biophys.*, 1973, **158**, 288.

deoxy- and unsaturated derivatives thereof,[752] and benzyl D-glucopyranosides carrying substituent benzyl, trityl, and allyl groups.[160]

High-resolution field-desorption mass spectrometry has been applied to the disodium salts of D-glucose 6-phosphate and to 2-, 3-, and 4-monofluoro-derivatives thereof.[753] The benzeneboronates of a number of simple methyl hexopyranosides have also been examined.[259]

More complex glycosides that have been examined include flavonoid mono- and di-glycosides (as the methyl ethers),[754] cerebrosides (as the TMS derivatives),[755] modified maltose acetates,[756] D-fructosyl disaccharides,[757] and tri-, tetra-, and penta-saccharides as the acetylated *N*-arylglycosylamine derivatives.[758] In the latter instance, it was possible to determine the molecular weights of the oligosaccharides, and the nature and sequence of the constituent sugars.

The mass spectra of *N*-salicylidene derivatives of amino-sugars[339] and amino-glycoside antibiotics[560] have been studied in detail; each of the spectra showed a strong peak corresponding to the molecular ion.

Acidic compounds to be studied included D-glucuronic acid (as the TMS derivative),[759] benzodiazepin D-glucuronides,[760] drug D-glucuronides,[761] and *N*-acetylneuraminic acid (as the peracetylated methyl ester methyl glycoside).[762]

Among compounds of biological interest to be examined were the *C*-methylinositols (stereoisomers can be characterized),[533] derivatives of kanamycin A,[550] methylated nucleosides,[763] TMS derivatives of a series of anhydropurine nucleosides (8,2′- and 8,3′-linked isomers can be distinguished),[764] a wide variety of derivatives of 6,5′-anhydropyrimidine nucleosides,[765] and various 7- and 9-β-D-ribofuranosylpurines.[766] Chemical ionization methods used in the latter study revealed that 7-bonded isomers are more susceptible to cleavage of the glycosidic bond; since protonated

[752] J. Mitera, V. Kubelka, A. Zobacova, and J. Jary, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3744.
[753] H.-R. Schulten, H. D. Beckey, E. M. Bessell, A. B. Foster, M. Jarman, and J. H. Westwood, *J.C.S. Chem. Comm.*, 1973, 416.
[754] H. Wagner and O. Seligmann, *Tetrahedron*, 1973, **29**, 3029.
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[763] D. L. von Minden and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1973, **95**, 7480.
[764] D. C. K. Lin, L. Slotin, K. K. Ogilvie, and J. B. Westmore, *J. Org. Chem.*, 1973, **38**, 1118.
[765] E. G. Lovett and D. Lipkin, *J. Amer. Chem. Soc.*, 1973, **95**, 2312.
[766] J. A. McCloskey, J. H. Futrell, T. A. Elwood, K. H. Schram, R. P. Panzica, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1973, **95**, 5762.

species are involved, the reaction can be likened to the acid-catalysed hydrolysis of these nucleosides. TMS derivatives of various sugar phosphates have also been subjected to mass-spectroscopic examination.[238]

X-Ray Crystallography

Last year it was noted that, for the period 1967—1972, the number of crystal structures of carbohydrates reported annually rose from approximately five to forty. Fifty or so new crystal structures were published during 1973, continuing the upward trend in the use of *X*-ray methods.

The occurrence of intramolecular hydrogen bonding in crystals of mono- and di-saccharides has been reviewed,[767] and the following crystal and molecular structures have been reported (solvents of crystallization are not included).

Simple Monosaccharide Derivatives.—α-D-Glucopyranose (refined),[768] α-D-xylopyranose,$CaCl_2$,[769] α-D-galactopyranose,$CaBr_2$,[770] 2-acetamido-2-deoxy-α-D-galactopyranose,[771] methyl 3,6-anhydro-α-D-glucopyranoside,[187] 2,6-anhydro-β-D-fructofuranose,[772] 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose,[773] methyl 1-thio-α-D-ribopyranoside ($^1C_4$),[774] methyl 5-thio-α- and -β-D-ribopyranosides ($^4C_1$),[775] methyl 1,5-dithio-α- and -β-D-ribopyranosides ($^1C_4$ and $^4C_1$, respectively),[776] methyl 6-*O*-acetyl-β-D-galactopyranoside (to ascertain the conformation about the C-5–C-6),[777] 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-*exo*-ethoxyethylidene)-α-D-glucopyranose,[707] 5-*O*-chloroacetyl-1,2:3,4-di-*O*-isopropylidene-α-D-glucoseptanose,[697] methyl 2,6-dichloro-2,6-dideoxy-3,4-*O*-isopropylidene-α-D-altropyranoside,[708] and scillicyanoside (a steroidal glycoside).[778]

Acid Derivatives.—2,3,6-Tri-*O*-methyl-D-galactono-1,4-lactone,[779] and *N*-acetylneuraminic acid [780] and its methyl ester.[781]

Di- and Tri-saccharides.—Sucrose [782] (refined, and compared with refined, neutron-diffraction measurements [782a]), lactose,$CaCl_2$,[783] lactose,$CaBr_2$,[784]

[767] G. A. Jeffrey, *Carbohydrate Res.*, 1973, **28**, 233.
[768] E. Hough, S. Neidle, D. Rogers, and P. G. H. Troughton, *Acta Cryst.*, 1973, **B29**, 365.
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[774] R. L. Girling and G. A. Jeffrey, *Acta Cryst.*, 1973, **B29**, 1006.
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[781] A. M. O'Connell, *Acta Cryst.*, 1973, **B29**, 2320.
[782] J. C. Hanson, L. C. Sieker, and L. H. Jensen, *Acta Cryst.*, 1973, **B29**, 797.
[782a] G. M. Brown and H. A. Levy, *Acta Cryst.*, 1973, **B29**, 790.
[783] W. J. Cook and C. E. Bugg, *Acta Cryst.*, 1973, **B29**, 907.
[784] C. E. Bugg, *J. Amer. Chem. Soc.*, 1973, **95**, 908.

lactobionic acid,$CaBr_2$,[785] β-D-xylobiose hexa-acetate,[786] 6-*O*-α-D-glucopyranosyl-D-fructofuranose (isomaltulose),[787] melezitose,[788] and *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose.[789]

Nucleosides and their Derivatives and Analogues.—5-Chloro-2′-deoxyuridine,[790] 2′-deoxyuridine 5′-phosphate,[791] 5-hydroxyuridine,[792] 3-deazauridine,[793] (+)-2′-*O*-tetrahydropyranyluridine,[794] 1-β-D-arabinofuranosyluracil.[795] The following anhydrouracil derivatives were also examined: 2,2′-anhydrouridine,[796] 2,2′-anhydro-1-β-D-arabinofuranosyluracil,[797, 798] and 2,5′-anhydro-2′,3′-*O*-isopropylideneuridine.[798] Also 1-β-D-arabinofuranosylthymine,[799] 1-β-D-arabinofuranosylcytosine [800] and the hydrochloride [801] and 2,2′-anhydro-derivative thereof,[802] cytidine 2′,3′-cyclic phosphate,[803] and adenosine hydrochloride.[804]

Crystal and molecular structures have been reported for the following nucleosides containing unnatural bases: 1-β-D-ribofuranosylimidazole,[805] 2-chloro-1-β-D-ribofuranosylbenzimidazole,[806] 2-thio-1-β-D-ribofuranosylbenzimidazole,[807] and 2-(4-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enopyranosyl)-5,6-dichlorobenzotriazole.[808]

The conformations of dinucleoside phosphates and nucleotides have been considered on the basis of structures determined by *X*-ray methods and energy calculations.[809]

Antibiotics.—Tubercidin [810, 810a] and formycin.[811]

[785] W. J. Cook and C. E. Bugg, *Acta Cryst.*, 1973, **B29**, 215.
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[796] D. Suck and W. Saenger, *Acta Cryst.*, 1973, **B29**, 1323.
[797] L. T. J. Delbaere and M. N. G. James, *Acta Cryst.*, 1973, **B29**, 2905.
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[803] C. L. Coulter, *J. Amer. Chem. Soc.*, 1973, **95**, 570.
[804] K. Shikata, T. Ueki, and T. Mitsui, *Acta Cryst.*, 1973, **B29**, 31.
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[806] S. Sprang and M. Sundaralingam, *Acta Cryst.*, 1973, **B29**, 1910.
[807] P. Prusiner and M. Sundaralingam, *Acta Cryst.*, 1973, **B29**, 2328.
[808] J. Lopez de Lerma, S. Martínez-Carrera, and S. García-Blanco, *Acta Cryst.*, 1973, **B29**, 537.
[809] S.-H. Kim, H. M. Berman, N. C. Seeman, and M. D. Newton, *Acta Cryst.*, 1973, **B29**, 703.
[810] R. M. Stroud, *Acta Cryst.*, 1973, **B29**, 690.
[810a] J. Abola and M. Sundaralingam, *Acta Cryst.*, 1973, **B29**, 697.
[811] P. Prusiner, T. Brennan, and M. Sundaralingam, *Biochemistry*, 1973, **12**, 1196.

Cyclitol Derivatives.—*myo*-Inositol,$MgCl_2$,[812] *myo*-inositol,$CaBr_2$,[813] 2-acetoxymethyl-1,3,4,6-tetra-*O*-acetyl-*epi*-inositol,[814] and 1,2:5,6-di-*O*-isopropylidene-3,4-di-*O*-toluene-*p*-sulphonyl-L-*chiro*-inositol.[815]

Miscellaneous Structures.—The crystal structure of the perchlorate of the acetoxonium ion formed from pinacol acetate showed that the five-membered ring is planar, with the isolated methyl group also in this plane.[816] This indicates that, in the crystal at least, mesomeric stabilization of the ion outweighs non-bonded repulsions associated with the eclipsed methyl groups.

[812] G. Blank, *Acta Cryst.*, 1973, **B29**, 1677.
[813] W. J. Cook and C. E. Bugg, *Acta Cryst.*, 1973, **B29**, 2404.
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25
Polarimetry

Although polarimetry (both monochromatic and spectral) remains a valuable tool in carbohydrate chemistry, few papers have appeared on this subject over the past year.

The absolute configurations of sugars and partially methylated sugars can be determined by c.d. measurements on the derived alditol acetates at 213 nm, the wavelength at which the carbonyl groups are chromophoric. The method requires only milligram quantities of the acetates, and is therefore suitable for use in conjunction with g.l.c.[817]

Cotton effects have been observed for a series of aldose dithioacetals.[818] For ethylene dithioacetals, the sign of the Cotton effect of longest wavelength is apparently related to the configuration at C-3 of the parent carbohydrate, whereas, for diethyl dithioacetals, Cotton effects observed at wavelengths below 220 nm appear to be related to the configurations at C-2–C-4; for example, if the configuration at these centres is DLD, the effect is positive.

C.d. measurements have been used, in conjunction with n.m.r. spectroscopy, to investigate the stereochemistry at the acetal centre of 2′,3′-*O*-benzylidenated nucleosides.[207] Extended Hückel methods applied to whole molecules have been used to calculate c.d. curves for adenine nucleosides in a variety of conformations. Substitution and puckering of the sugar ring have been shown to be less significant than the sugar–base torsional angle, and $n \rightarrow \pi^*$ transitions were concluded to be as important quantitatively as $\pi \rightarrow \pi^*$ transitions.[819] C.d. and magnetic c.d. studies of guanosine and several of its derivatives have been reported, and it was concluded that the c.d. spectra are very sensitive to changes of conformation [820] (see above). Related studies have been carried out on pyrimidine nucleosides and 2-thio-analogues thereof, and the results of c.d. spectroscopy were considered in conjunction with n.m.r. spectroscopic measurements.[821]

[817] G. M. BeBault, J. M. Berry, Y. M. Choy, G. G. S. Dutton, N. Funnell, L. D. Hayward, and A. M. Stephen, *Canad. J. Chem.*, 1973, **51**, 324.
[818] M. K. Hargreaves and D. L. Marshall, *Carbohydrate Res.*, 1973, **29**, 339.
[819] C. A. Bush, *J. Amer. Chem. Soc.*, 1973, **95**, 214.
[820] J.-M. Delabar and W. Guschlbauer, *J. Amer. Chem. Soc.*, 1973, **95**, 5729.
[821] G. Cleve, G.-A. Hoyer, G. Schulz, and H. Vorbrüggen, *Chem. Ber.*, 1973, **106**, 3062.

26
Separatory and Analytical Methods

Chromatographic Methods

Gas–Liquid Chromatography.—A review (in Japanese) has appeared on the applications of g.l.c. in biological systems.[822]

Trimethylsilyl (TMS) ethers continue to find favour as derivatives for g.l.c. analyses; such ethers of *O*-methyl oximes of aldoses, ketoses, and their deoxy and *O*-methyl derivatives have been separated and examined by mass spectrometry, although a separation of *syn*- and *anti*-isomers was not always achieved.[823] Further reports on the analysis of TMS ethers of mono- and di-saccharides[824] and flavonoid glycosides[825] by g.l.c. have appeared, and a particularly interesting application has been to the TMS derivatives of various aldose and ketose mono- and di-phosphates.[238] The mass spectra of these volatile derivatives were also examined.

Acetates of 2-amino-2,6-dideoxyhexitols have been examined,[826] and acetylated aldononitriles (prepared by dehydration of the oximes) have proved popular for the analysis of sugars in polysaccharide hydrolysates.[827–829] Satisfactory separations of trifluoroacetates of cyclitols[830] and methyl glycosides[831] have also been achieved.

Di-, tri-, and tetra-*O*-methyl ethers of methyl α-D-mannopyranoside have been fractionated,[832] and permethylated alditols and aldonates have been used in analyses of free sugars and sugar acids.[833]

Butyl- and benzene-boronates of aldoses have proved amenable to separation by g.l.c.[834]

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[834] R. Greenhalgh and P. J. Wood, *J. Chromatog.*, 1973, **82**, 410.

Column and Ion-exchange Chromatography.—Anomeric aryl glycosides and 1-thio-glycosides have been shown to be separable on a cation-exchange column,[835] and mixtures of cyclitols can be separated using either cation- or anion-exchangers.[836] An authoritative review has appeared on the separation on resins of saccharinic, aldonic, and alduronic acids,[837] and a paper specifically on the separation of aldonic acids has been published.[838]

Free sugars can be separated using ion-exchange resins in the bisulphite form, and the resolutions achieved have been studied as a function of temperature.[839] Poly(4-vinylbenzeneboronic acid) has been used as the stationary phase,[40] and 'boric-acid gels' have also been employed,[840] in fractionations of mono- and oligo-saccharides. Bio-Gel P-4 has been applied to the separation (by gel filtration) of partially methylated mono- and oligo-saccharides,[841] and also of higher oligomers (DP 10—15) of D-glucose.[841a]

Paper Chromatography and Electrophoresis.—Recommendations have been made concerning the publication of chromatographic data to assist in comparison of paper-chromatographic (and t.l.c.) results obtained in different laboratories.[842]

Paper-chromatographic data on free sugars and *N*-acetamido-compounds have been reported,[826] and the products (L-gulonic acid and its 1,4-lactone, D-glucaric acid and its 6,3- and 1,4-lactones and 1,4:6,3-dilactone, L-ascorbic acid, and *myo*-inositol) of metabolism of D-glucuronic acid by mammals have been separated by multiple-elution procedures.[843] Data on the thin-layer and paper-chromatographic separations of a series of alkyl 1-thio-β-D-galactopyranosides have also appeared.[844]

Sucrose can be detected on paper chromatograms by spraying with invertase, followed by treatment with such conventional reagents as alkaline silver nitrate.[845]

The paper-electrophoretic mobilities of various monosaccharides and methyl ethers thereof, disaccharides, glycosides, and sugar acids have been examined in both glycerol–boric acid and sulphonated benzeneboronic acid buffers.[846] In most cases, the two media gave similar separations, but glycerol–boric acid has the advantages of being more readily prepared, giving higher absolute mobilities, and sharper spots after spraying.

[835] J. Schneider and Y. C. Lee, *Carbohydrate Res.*, 1973, **30**, 405.
[836] E. Päärt and O. Samuelson, *J. Chromatog.*, 1973, **85**, 93.
[837] P. Jandera and J. Churáček, *J. Chromatog.*, 1973, **86**, 351.
[838] J. Havlicek and O. Samuelson, *J. Chromatog.*, 1973, **83**, 45.
[839] Y. Takasaki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2575.
[840] K. Reske and H. Schott, *Angew. Chem. Internat. Edn.*, 1973, **12**, 417.
[841] E. Grellert and C. E. Ballou, *Carbohydrate Res.*, 1973, **30**, 218.
[841a] N. K. Sabbagh and I. S. Fagerson, *J. Chromatog.*, 1973, **86**, 184.
[842] I. Smith, A. D. Baitsholts, A. A. Boulton, and K. Randerath, *J. Chromatog.*, 1973, **82**, 159.
[843] E. Puhakainen and O. Hanninen, *Acta Chem. Scand.*, 1972, **26**, 3599.
[844] V. Pačes and J. Frgala, *J. Chromatog.*, 1973, **79**, 373.
[845] K. K. Schlender and J. A. Levin, *Analyt. Biochem.*, 1973, **52**, 630.
[846] B. Pettersson and O. Theander, *Acta Chem. Scand.*, 1973, **27**, 1900.

Thin-layer Chromatography.—A number of applications of t.l.c. have been noted in the preceding section. T.l.c. has also been applied to 6-deoxyhexoses,[847] gentamicin C, and other amino-glycoside antibiotics,[848] and polyaromatic D-glucopyranosides.[849]

Other Analytical Methods.—Ethanolic aniline malonate is claimed to be superior to the more commonly used aniline hydrogen phthalate for the quantitative analysis of sugars.[850] Fluorometric methods have been recommended for simple sugars [851] and amino-sugars,[852] and hexosamines can also be determined by treatment with hot alkali, followed by spectrophotometric analysis at 302 nm.[853]

Colorimetric methods for determining di- and oligo-saccharides in the presence of monosaccharides,[854] and for neutral and acidic sugars,[855] and new titrimetric methods for uronic acids [856] and for L-ascorbic acid [857] have been reported.

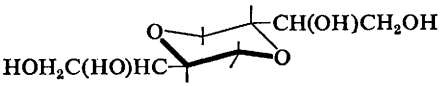
Specific analytical procedures for D-fructose (enzymic) [858] and evernimicin D (densitometric) [859] have also appeared.

[847] S. Singh and B. E. Stacey, *Analyst*, 1972, **97**, 977.
[848] W. L. Wilson, G. Richard, and D. W. Hughes, *J. Chromatog.*, 1973, **78**, 442.
[849] J. F. Lawrence and R. W. Frei, *J. Chromatog.*, 1973, **79**, 223.
[850] G. Caldes and B. Prescott, *J. Chromatog.*, 1973, **84**, 220.
[851] S. Honda, K. Kakimoto, K. Kakehi, and K. Takiura, *Analyt. Chim. Acta*, 1973, **64**, 310.
[852] M. Roth and A. Hampai, *J. Chromatog.*, 1973, **83**, 353.
[853] J. Lewandowski, *Analyt. Biochem.*, 1973, **54**, 325.
[854] R. M. McCready and E. D. Ducay, *Carbohydrate Res.*, 1973, **28**, 100.
[855] N. B. Patil, S. V. Bhide, and N. R. Kale, *Carbohydrate Res.*, 1973, **29**, 513.
[856] N. Blumenkrantz and G. Asboe-Hansen, *Analyt. Biochem.*, 1973, **54**, 484.
[857] M. Z. Barakat, S. K. Shehab, N. Darwish, and A. El-Zoheiry, *Analyt. Biochem.*, 1973, **53**, 245.
[858] B. Sabater and C. Asensio, *Analyt. Biochem.*, 1973, **54**, 205.
[859] P. Kabasakalian and S. Kalliney, *J. Chromatog.*, 1973, **86**, 145.

27
Alditols

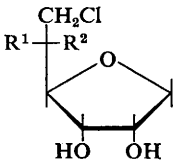
Glycerol, erythritol, arabinitol, and xylitol have been isolated from an unfermented extract of *Cannabis sativa* L.,[7,8] while 1-*O*-β-D-fructofuranosyl-D-mannitol, 5-*O*-β-D-fructofuranosyl-D-arabinitol, 1,6-di-*O*-β-D-fructofuranosyl-D-mannitol, 1,5-di-*O*-β-D-fructofuranosyl-D-arabinitol, and 1-*O*-β-D-fructofuranosyl-6-*O*-[β-D-fructofuranosyl-(2 → 6)-β-D-fructofuranosyl]-D-mannitol were among several unusual oligosaccharides found in the honeydew of *Sphacelia sorghi* (a parasitic fungus), which also contains D-arabinitol and D-mannitol.[9]

Free radicals formed on γ-irradiation of crystalline alditols have been investigated by e.s.r. spectroscopy.[860] In addition to 1,4-anhydroerythritol, acid-catalysed dehydration of erythritol has been shown to yield *trans*-2,5-di-(1,2-dihydroxyethyl)-1,4-dioxan (403) by removal of water from two



(403)

molecules of the tetritol.[861] Allitol reacted with fuming hydrochloric acid at 100 °C to give 1,4-anhydro-5,6-dichloro-5,6-dideoxy-DL-talitol (404) and 1,4-anhydro-6-chloro-6-deoxy-DL-allitol (405); the corresponding bromo-compounds were obtained from a similar reaction with an excess of hydrogen bromide, and possible mechanisms for formation of the products were



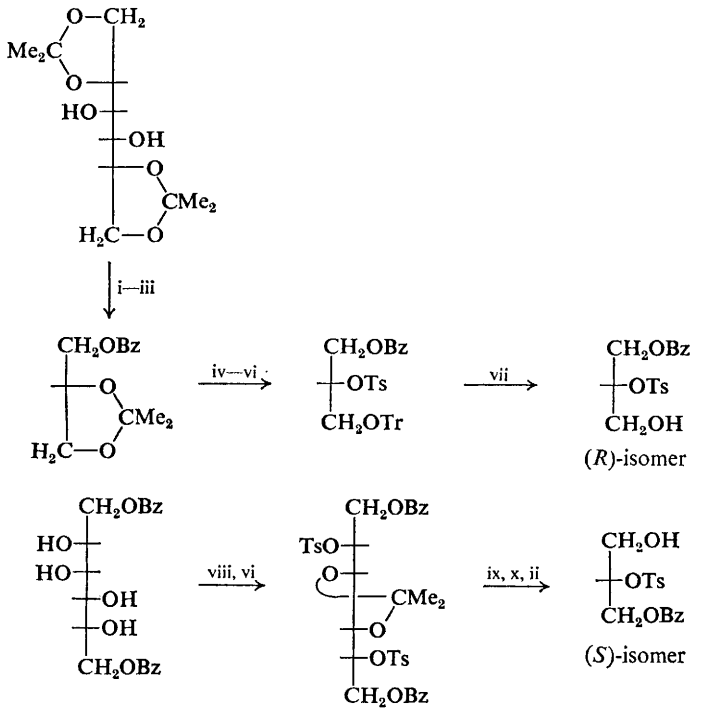
(404) R^1 = H; R^2 = Cl
(405) R^1 = OH; R^2 = H

[860] I. V. Nikitin, I. V. Miroshnichenko, L. I. Kudryashov, M. E. Dyatkina, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 356 (*Chem. Abs.*, 1973, **78**, 72 484u).

[861] A. H. Haines and A. G. Wells, *Carbohydate Res.*, 1973, **27**, 261.

discussed.[862] Vinyl ethers of 1,4:3,6-dianhydro-D-glucitol and -mannitol have been prepared, and were readily polymerized in the presence of boron trifluoride.[166]

A detailed study of the sulphonylation of 2,4-*O*-methylenexylitol has been reported.[863] 1,2:5,6-Di-*O*-isopropylidene- and 1,6-di-*O*-benzoyl-D-mannitol have been converted into optically active derivatives of glycerol by the transformations shown in Scheme 144.[864] A stereospecific synthesis

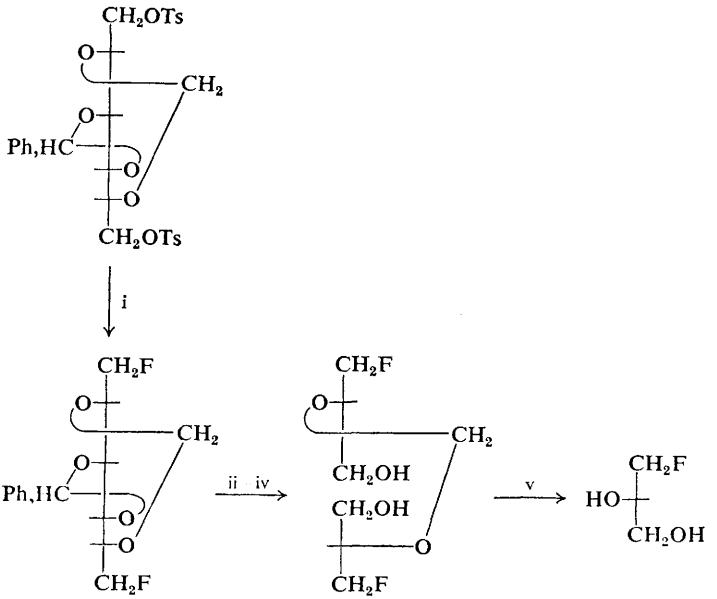


Reagents: i, $Pb(OAc)_4$; ii, $NaBH_4$; iii, BzCl–py; iv, 0.5M-HCl; v, TrCl–py; vi, TsCl–py; vii, H_2–Pd; viii, Me_2CO–$CuSO_4$; ix, AcOH–HCl; x, $NaIO_4$

Scheme 144

of 1-deoxy-1-fluoro-L-glycerol from a D-mannitol derivative has been achieved by way of a fluoride-ion displacement (Scheme 145).[283] Syntheses of 1-*O*-methyl-D-erythritol, 1-*O*-methyl-L-threitol, 1,4-di-*O*-methylerythritol, and 1,4-di-*O*-methyl-L-threitol have been described; these methylated tetritols were required as reference compounds for a modified Smith

[862] J. M. Ballard and B. E. Stacey, *Carbohydrate Res.*, 1973, **30**, 83.
[863] G. M. Zarubinskii and S. N. Danilov, *J. Gen. Chem.* (*U.S.S.R.*), 1972, **42**, 2750.
[864] J. Y. Rouault, C. Morpain, M. Tisserand, and E. Cerutti, *Compt. rend.*, 1973, **277**, *C*, 787.

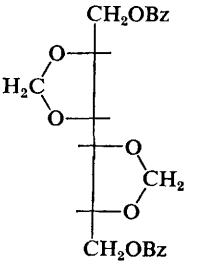


Reagents: i, Bu_4NF–MeCN; ii, H_2–Pd; iii, $NaIO_4$; iv, KBH_4; v, MeOH–HCl

Scheme 145

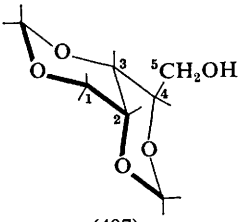
degradation of polysaccharides that involved methylation prior to acid hydrolysis.[159a]

A new method of preparing methylene acetals has been referred to already in Chapter 5; for example, treatment of 1,6-di-*O*-benzoyl-D-mannitol with NBS in DMSO afforded the diacetal (406).[197] There are

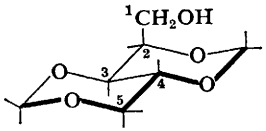


(406)

important consequences for carbohydrate chemistry in the observation that vicinal oxygen substituents preferentially adopt a *gauche* arrangement in solution. Thus, formation of the *cis*-fused 1,3:2,4-diacetal (407), in preference to the *trans*-fused 2,4:3,5-diacetal (408), on condensation of



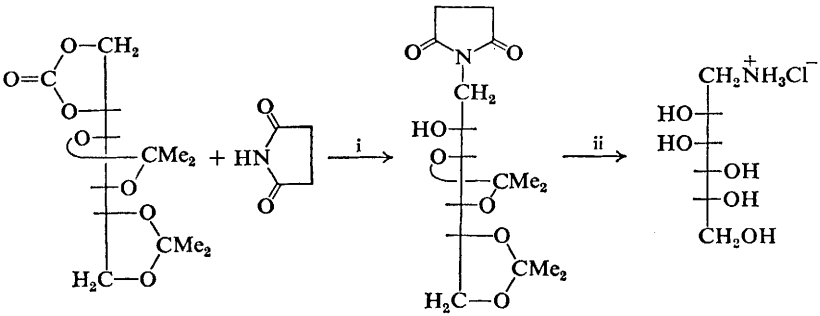
(407)



(408)

D-arabinitol with formaldehyde under conditions of equilibration is explained by the fact that the *cis*-fused system can adopt a conformation in which only *gauche* oxygen–oxygen interactions are present.[865] The broader implications for carbohydrate chemistry of the orientations of unshared electron pairs and polar bonds on adjacent pyrimidal atoms are discussed in a recent review.[866]

A new method for the synthesis of alditol derivatives of heterocyclic compounds has utilized a condensation of 1,2:3,4-di-*O*-isopropylidene-D-mannitol 5,6-cyclic carbonate with such bases as theophylline, 6-(benzylamino)purine, phthalimide, and succinimide (see Scheme 146) in the



Reagents: i, $Et_4N^+Br^-$ (*ca.* 155 °C); ii, MeOH–HCl

Scheme 146

presence of tetraethylammonium bromide.[867] 1-Deoxy-1-(indol-1-yl)-D-galactitol and -glucitol have been prepared,[378] and 1,1-diaryl derivatives of polyhydroxy-compounds resulted when monosaccharides reacted with aromatic hydrocarbons in the presence of aluminium trichloride.[868]

[865] L. Phillips and V. Wray, *J.C.S. Chem. Comm.*, 1973, 90.
[866] J. F. Stoddart in M.T.P. Internat. Review of Science, Series 1, Vol. 7, 'Carbohydrates', ed. G. O. Aspinall, Butterworths, London, 1973, p. 1.
[867] H. Komura, T. Yoshino, and Y. Ishido, *Carbohydrate Res.*, 1973, **31**, 154.
[868] M. Leonte and M. Stoica, *Steroids Lipids Res.*, 1970, **4**, 175 (*Chem. Abs.*, 1972, **77**, 152 468m).

Part II

MACROMOLECULES

1
Introduction

The main objectives of Part II remain unchanged from those of previous Reports in this series.

The naming of microbial organisms continues to present problems, particularly in view of the re-classification and re-naming advocated in the interests of taxonomy. The author's designation of the organism has generally been used in this Report, but readers are advised to look for references under all possible names for an organism. The common, trivial names for plants have usually been given in addition to the Latin names. However, it should be remembered that more than one trivial name is often used for the same species.

It is now apparent that many enzymes contain carbohydrate moieties and consequently are glycoproteins. However, all reports dealing with enzymes are included in Chapter 6 for convenience. α-Lactalbumin and related macromolecules have been assigned to various chapters along the lines indicated in Volume 5, Part II, Chapter 1. The reports in Chapter 6 include references to a number of new enzymes (including glycosidases) specific for glycolipids.

The publication during 1973 of the Recommendations of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry on Enzyme Nomenclature has permitted further classification of enzymes. In addition to those cited in previous Reports, trivial and systematic names and E.C. numbers have now been recommended for many other enzymes; wherever possible, this Report has employed the trivial names recommended. One of the outcomes of the new classification is that many divisions of enzymes previously suspected have now been formalized. The reader's attention is drawn to the fact that the name of an enzyme used in previous Reports may, in the light of new evidence, have applied to a family of enzymes; it may have been necessary, however, to retain and apply the name to only one species within the family. Certain names of enzymes have been changed completely by the new Recommendations and, in these cases, both old and new names are given in the interests of clarity and continuity. In some cases, the E.C. number of an enzyme has been deleted in the new Recommendations, but it has been necessary to retain these numbers (names) in a few cases in order to accommodate references for which it has been impossible to determine the appropriate new category.

In addition to the re-naming and re-classification of enzymes, the format of Chapter 6 has been modified slightly. Glycohydrolases active towards glycosides are again dealt with first, but are now followed by oligosaccharidases and polysaccharidases (including eliminases) arranged collectively and in alphabetical order. Enzymes with activities other than of a glycolytic nature are dealt with subsequently. Assignment of an enzyme to a particular section is based primarily on information given in the paper, but it must be recognized that, for example, various galactosidases are also active against rhamnosides. References to glycohydrolases active towards a unique glycosidic substrate have been included in the relevant general section: for example, galactosidases active against glycolipids are covered under galactosidases, and quercitrinases are covered under rhamnosidases. Despite the distinction in the new Recommendations between β-acetamidodeoxyhexosidases and β-acetamidodeoxy-glucosidases and -galactosidases, all references to these enzymes have been included in one section, since it was not always possible to determine from the information available whether the author's designation is correct.

With continued discoveries, it is inevitable that not all enzymes have been accorded an E.C. number; a pertinent problem in this respect lies with glucanases, *etc.* Where it has not been possible to assign an E.C. number, the reference has been placed in an appropriate miscellaneous section (for example, Miscellaneous Glucanases). The term 'amylase' has been used when the type of amylase was not specified or when the combined α- and β-amylase activities were measured. In many cases, however, the problem of identifying an enzyme could be resolved if the author(s) recorded the E.C. number.

Tables have again been used in Chapter 8 (Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, Gangliosides, and Glycolipids) to facilitate easy reference to both enzymes and carbohydrate-containing macromolecules derivatized by insolubilization and to the use of cyclic imidocarbonate derivatives of macroporous agarose as a matrix for insolubilization. Use of the prefix 'tri' to denote the substitution of hexoglycans has been discontinued in this Chapter, since it is often applied to situations where the degree of substitution is less than three; for example, 'cellulose triacetate (d.s. 2.3)'. The prefix 'per' is applied only in the proper sense of the term. Since agarose is now marketed in macroporous form by a number of companies, it is referred to as such in Chapter 8 and the use of the trade-name 'Sepharose' has been discontinued. However, the name 'Sephadex' has been retained, since it refers to a particular type of dextran derivative.

2
General Methods

BY R. J. STURGEON

Analysis

Analytical methods for carbohydrates have been dealt with in a treatise which includes chapters on sugar analysis of cells in culture by isotope dilution techniques, methylation analysis of polysaccharides, g.l.c. of carbohydrates as butane boronic esters, isolation and characterization of glycosphingolipids, release of oligosaccharides from glycolipids, analysis of sugars by automated liquid chromatography, determination of the molecular weights of glycoproteins on polyacrylamide gels, a study of carbohydrates in glycoproteins, the assays of 2-acetamido-2-deoxygalactitol and unsaturated amino-acids produced by β-elimination reactions in glycoproteins, and the determination of sialic acid.[1] Another treatise has reviewed polysaccharide methodology with respect to such analytical methods as paper chromatography, g.l.c.–m.s., ^{1}H n.m.r. spectroscopy, fragmentation procedures such as glycol cleavage (including the Smith degradation), and methylation techniques.[2] A review has appeared on the g.l.c. of carbohydrates in food with special reference to TMS derivatives.[3]

Optimum concentrations of thymol and Fe^{3+} have been sought for the determination of neutral and acidic carbohydrates in microgram quantities. On the basis of the molar extinction coefficients of the coloured complexes formed by D-glucose, sucrose, and D-arabinose, the reagent compared well with the phenol–sulphuric acid reagent.[4] Bacteriostatic agents such as sodium azide and benzoic acid have been reported to interfere with the development of the specific colour produced by carbohydrates with anthrone reagents.[5] The stable fluorescence produced when ethylenediamine sulphate is heated with aqueous solutions of D-glucose has been used in a paper chromatographic method for the detection of reducing sugars and polyhydroxy-compounds.[6] A modified periodate-oxidation

[1] 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1973, Vol. 28B.
[2] G. O. Aspinall and A. M. Stephen, in 'Carbohydrates', ed. G. O. Aspinall, in MTP International Review of Science, Organic Chemistry Series One', Butterworths, London, 1973, Vol. 7, p. 285.
[3] G. G. Birch, *J. Food Technol.*, 1973, **8**, 229.
[4] N. B. Patil, S. V. Bhide, and N. R. Kale, *Carbohydrate Res.*, 1973, **29**, 513.
[5] H. K. Tong, K. H. Lee, and H. A. Wong, *Analyt. Biochem.*, 1973, **51**, 390.
[6] S. Honda, K. Kakimoto, K. Kakehi, and K. Takiura, *Analyt. Chim. Acta*, 1973, **64**, 310.

procedure has been reported to be suitable for the routine determination of a wide range of carbohydrates, including soluble sugars, sugar alcohols, oligosaccharides, and hydrolysis products of polysaccharides, in solution.[7] The fluorescence of a lanthanum chelate in diethanolamine had been used in a specific method for the colorimetric and fluorometric determination of reducing carbohydrates.[8] A comparative study of certain aniline salts used in the detection of carbohydrates by spot-elution chromatography has indicated that the best results are obtained using aniline malonate.[9] Owing to the relative differences in the molar extinction coefficients at the wavelengths concerned, the measurement of ferrocyanide, rather than ferricyanide, has provided an increase in sensitivity in the determination of reducing sugars in the nanomole range.[10] A specific method for the qualitative and quantitative analysis of reducing carbohydrates has been reported; it involves measurement of the amount of tritium found following reduction of the sugars with sodium borotritide and separation on ion-exchange papers.[11] A similar method has been applied to the determination of reducing sugars, oligosaccharides, and glycoproteins.[12]

The qualitative determination of cerebrosides, after their separation by t.l.c., has been achieved by use of an anthrone spray-reagent.[13] The percentage distribution of *N*-acetylneuraminic acid in individual gangliosides has been determined by a densitometric method that required their separation by t.l.c. and reaction with a resorcinol reagent.[14] The ability of a cationic carbocyanine dye to form with lipopolysaccharides aggregates having different absorption maxima at different molar concentrations and in different solvents has been used in the colorimetric analysis of these polymers. Although the time and the temperature of incubation of the lipopolysaccharide–dye complexes are important variables, having marked, but different, effects on lipopolysaccharides from various sources, concentration-dependent standard curves were established for the lipopolysaccharides.[15] A quantitative determination of acid mucopolysaccharides has been based on the interaction of these macromolecules with the zirconyl ion. The method involves the determination of the polymer molecules rather than their hydrolytic components.[16] Serum acid mucopolysaccharides have been digested by proteolytic enzymes and precipitated by methanol and detergents prior to colorimetric analysis by the carbazole reaction. The higher values obtained, compared with similar methods, are possibly due to elimination of the loss of mucopolysaccharide by omitting

[7] A. E. Flood and C. A. Priestley, *J. Sci. Food Agric.*, 1973, **24**, 945.
[8] M. Lever, *Biochem. Med.*, 1973, **7**, 274.
[9] G. Caldes and B. Prescott, *J. Chromatog.*, 1973, **84**, 220.
[10] D. K. Kidby and D. J. Davidson, *Analyt. Biochem.*, 1973, **55**, 321.
[11] H. E. Conrad, E. Varboncouer, and M. E. James, *Analyt. Biochem.*, 1973, **51**, 486.
[12] C. McLean, D. A. Werner, and D. Aminoff, *Analyt. Biochem.*, 1973, **55**, 72.
[13] C. M. Van Gent, O. J. Roseleur, and P. Van der Bijl, *J. Chromatog.*, 1973, **85**, 174.
[14] F. Šmíd and J. Reinišová, *J. Chromatog.*, 1973, **86**, 200.
[15] P. Zey and S. Jackson, *Appl. Microbiol.*, 1973, **26**, 129.
[16] A. H. Wardi and G. A. Michos, *Analyt. Biochem.*, 1973, **51**, 274.

the use of trichloroacetic acid and dialysis in removal of protein.[17] A quantitative scanning of glycoproteins on polyacrylamide gels has been achieved by staining with the periodic acid–Schiff reagent.[18] The staining properties of Aniline Blue and primuline with isolated preparations of variously linked glucans have been investigated. Contrary to other reports, neither fluorochrome is specific for β-(1 → 3)-linked D-glucose polymers.[19] Pentosans in cereals have been converted into furfural by hydrochloric acid; colorimetric determination of the furfural was simplified by stabilization of the aniline–furfural complex with buffers.[20]

Neutral sugars have been released as their methyl glycosides from glycoproteins and mucopolysaccharides. After removal of amino-acids and amino-sugars by ion-exchange chromatography, the glycosides were hydrolysed to the free sugars and transformed into the aldononitrile acetates prior to quantitative determination by g.l.c.[21] The method has also been used for the determination of neutral sugars in polysaccharides.[22] 2-Amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and sialic acid have been estimated in hydrolysates of bovine oestrus cervical glycoprotein as the *N*-ethoxycarbonylated derivatives.[23] Methanolysis using acidified [^{14}C]methanol, which avoids humin formation by decomposition of component residues, has formed the basis of a quantitative method of analysis of an extracellular microbial polysaccharide by counting the individual [^{14}C]methyl glycosides after separation by paper chromatography.[24] Columns of polyalcohol fatty-acid esters have been used for the separation of commonly occurring hexoses as their trifluoroacetyl derivatives.[25] Ozonolysis followed by treatment with alkali has been used to release oligosaccharides from glycosphingolipids in mammalian erythrocytes, after which the sugars were determined as the TMS ethers of the derived glycitols.[26]

An improved separation of monosaccharides has been achieved at elevated temperatures on ion-exchange resins using water as eluant.[27] The influence of the organic base counter-ions and their degree of substitution on the separation of carbohydrates on strongly acidic cation-exchange resins has been discussed. Contrary to previous findings with inorganic ions, increasing size of the ion reduced the capacity ratios for the carbohydrate. The trimethylammonium form of the resin was found to give the

[17] Y. Emura and T. Mukuda, *Seikagaku*, 1973, **45**, 30.
[18] J. M. Matthieu and R. H. Quarles, *Analyt. Biochem.*, 1973, **55**, 313.
[19] G. Faulkner, W. C. Kimmins, and R. G. Brown, *Canad. J. Botany*, 1973, **51**, 1503.
[20] J. Cerning and A. Guilbot, *Cereal Chem.*, 1973, **50**, 176.
[21] R. Varma, R. S. Varma, W. S. Allen, and A. H. Wardi, *J. Chromatog.*, 1973, **86**, 205.
[22] R. Varma, R. S. Varma, and A. H. Wardi, *J. Chromatog.*, 1973, **77**, 222.
[23] F. André, C. André, K. S. P. Bhushana Rao, P. L. Masson, and J. F. Heremans, *Carbohydrate Res.*, 1972, **25**, 395.
[24] P. A. Sandford, P. R. Watson, and A. R. Jeanes, *Carbohydrate Res.*, 1973, **29**, 153.
[25] S. Ando, T. Ariga, and T. Yamakawa, *J. Biochem. (Japan)*, 1973, **73**, 663.
[26] M. Ohashi and T. Yamakawa, *J. Lipid Res.*, 1973, **14**, 698.
[27] Y. Takasaki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2575.

best separation for a series of mono- and di-saccharides, which could then be determined quantitatively on a modified, moving-wire, flame-ionization detector.[28] Continuous development on thin-layer silica gel plates buffered with sodium phosphate has been used for the separation of rhamnose, quinovose, fucose, and glucose, the individual sugars being determined by the phenol–sulphuric acid method.[29] A simple, sensitive procedure for the detection of sucrose after paper chromatography has involved spraying the papers with a solution of invertase and then with silver nitrate reagents.[30] Similarly, weakly reducing malto-oligosaccharides have been determined by a combination of densitometry and treatment with glucoamylase-maltase. Determination of the time course and enzyme activity of the maltohexaose-producing amylase from *Aerobacter aerogenes* has been presented as an example of the use of this method.[31] An improved determination of known di- and oligo-saccharides in mixtures containing monosaccharides is based on colorimetric analysis of the mixture, using the phenol–sulphuric acid method, before and after reduction with sodium borohydride. A two-fold improvement over the hypobromite oxidation procedure, with no danger of destruction of sugars by over-oxidation, is claimed.[32] The quantitative estimation of sugars in blood and urine, following paper chromatographic separation and direct scanning of the sugars located by a colour reaction, has been discussed. Sources of error arising at different stages were analysed and controlled by modifications in the technique.[33] A paper chromatographic method suitable for the serial determination of carbohydrates from fresh, freeze-dried, or air-dried plants has been described.[34] Oligomeric aldonic acids (D-gluconic acid to cellohexaonic acid and D-xylonic acid to D-xylohexaonic acid) have been separated by ion-exchange chromatography in acetate and borate media. The logarithm of the distribution coefficient increased linearly with the degree of polymerization. A study of the influence of temperature showed that, for each additional sugar moiety, a constant incremental change is obtained not only in free energy (ΔG°) but also in enthalpy (ΔH°).[35] The separation of amino-sugars and amino-acids in glycoproteins and biological fluids has been achieved on resin-coated chromatoplates by using buffer solutions of different ionic strengths and pH values in the elution procedure.[36] [^{14}C]-Labelled lysine, hydroxylysine, and hydroxylysine glycosides have been separated on a single chromatographic column; the degree of glycosylation of the lysine and the ratio of disaccharide to monosaccharide could be measured during the same operation.[37] Improved

[28] J. S. Hobbs and J. G. Lawrence, *J. Chromatog.*, 1971, **72**, 311.
[29] S. Singh and B. E. Stacey, *Analyst*, 1972, **97**, 977.
[30] K. K. Schlender and J. A. Levin, *Analyt. Biochem.*, 1973, **52**, 630.
[31] S. Kobayashi, K. Kainuma, and S. Suzuki, *J. Jap. Soc. Starch Sci.*, 1972, **19**, 178.
[32] R. M. McCready and E. D. Ducay, *Carbohydrate Res.*, 1973, **28**, 100.
[33] I. S. Menzies, *J. Chromatog.*, 1973, **81**, 109.
[34] G. Hehl, *Z. analyt. Chem.*, 1973, **266**, 268.
[35] J. Havlicek and O. Samuelson, *J. Chromatog.*, 1973, **83**, 45.
[36] A. Hrabák, *J. Chromatog.*, 1973, **84**, 204.
[37] R. S. Askenasi and N. A. Kefalides, *Analyt. Biochem.*, 1972, **47**, 67.

resolution of the higher oligosaccharides of D-glucose has been obtained by means of gel-permeation chromatography on polyacrylamide gels having a higher exclusion limit than normal.[38] Thin-layer gel chromatography has been used to separate soluble dextran fractions of molecular weight up to 1×10^5 after combination with a triazine dye to render them visible during chromatography and to facilitate densitometric evaluation of the chromatograms.[39] A series of quaternary salts (octatrimethylammonium chloride to dodecyltriethylammonium bromide) have been synthesized and used to form complexes with borate and polysaccharides. Using this procedure, starch was selectively precipitated when present in solution with glycogen and eremuran.[40]

L-Galactose has been determined in the polysaccharides from flax seed, corn cob, corn root, and *Acer pseudoplatanus* by first oxidizing any D-galactose to D-galactonic acid with D-galactose dehydrogenase. The remaining sugars, including L-galactose, were converted into the TMS derivatives and estimated by g.l.c.[41] A method for the determination of D-fructose has been based on phosphorylation of the sugar by a highly specific fructokinase and coupling this reaction to NADP reduction by means of glucose phosphate isomerase and glucose 6-phosphate dehydrogenase. Owing to the lack of interference by other sugars, the procedure is appropriate for the determination of D-fructose in the presence of a high content of D-glucose.[42] The accuracy and precision of different methods for the determination of D-fructose in spermatozoa have been compared.[43] Statistical analysis showed that methods which use anthrone and anthrone plus thiourea give average values that vary from the theoretical by $\pm 25\%$. Regression and variance analyses showed that the diphenylamine, resorcinol, and enzymic methods were all equally suitable. It has been reported that in the use of the thioglycolic–sulphuric acid reagent for the determination of sedoheptulose, pentoses had almost no significant influence on the determination of sedoheptulose in the transketolase reaction.[44] A procedure for the fluorophotometric determination of amino-acids and amino-sugars is based on the production of highly fluorescent chelates by their interaction with pyridoxal and Zn^{2+} in pyridine–methanol solutions.[45] The characteristic absorption curve for amino-sugars, ranging from 225 to 360 nm with a maximum at 302 nm, has been utilized as a qualitative criterion for their identification in column effluents.[46] Several methods for trimethylsilylation of 2-amino-2-deoxyhexoses have been examined and conditions have been found for replacing one or both amino-protons by

[38] N. K. Sabbagh and I. S. Fagerson, *J. Chromatog.*, 1973, **86**, 184.
[39] P. T. Aspinall and J. N. Miller, *Analyt. Biochem.*, 1973, **53**, 509.
[40] B. N. Stepanenko and L. B. Uzdenikova, *Carbohydrate Res.*, 1972, **25**, 526.
[41] R. M. Roberts and E. Harrer, *Phytochemistry*, 1973, **12**, 2679.
[42] B. Sabater and C. Asensio, *Analyt. Biochem.*, 1973, **54**, 205.
[43] G. Peter and V. Havenstein, *Z. klin. Chem. klin. Biochem.*, 1972, **10**, 569.
[44] T. Ozawa, S. Saito, and I. Tomita, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2723.
[45] M. Maeda and A. Tsuji, *Analyt. Biochem.*, 1973, **52**, 555.
[46] J. Lewandowski, *Analyt. Biochem.*, 1973, **54**, 325.

TMS groups.[47] A doubling of the sensitivity in the estimation of sialic acid released in the neuraminidase assay has been ascribed to the efficiency of a dialysis step.[48] Uronic acids when heated with *m*-hydroxydiphenyl in sulphuric acid containing tetraborate produce a chromogen, the amount of which is proportional to the concentration of uronic acid.[49] The content of uronic acid in hemicelluloses has been determined after treatment of the polysaccharide in the following sequence: sodium borohydride, etherification with both propene oxide and diazomethane, reduction with sodium borotritide followed by acidic hydrolysis, and counting the tritiated D-glucose and 4-*O*-methyl-D-glucose by scintillation methods.[50] A rapid g.l.c. method for the analysis of mono- and di-saccharide mixtures has involved the preparation of the TMS ethers at mutarotational equilibrium.[51] An anthrone method has been reported for the differential determination of D-glucose and D-fructose occurring as free sugars or as constituents of sucrose or polymers.[52] A method employing g.l.c. of the TMS ethers has been described for quantifying small amounts of D-glucose and glycerol in agar culture media.[53] A new type of enzyme electrode has been constructed by placing a thin reaction layer containing a mixture of immobilized glucose oxidase and peroxidase over an iodide-sensitive membrane electrode. The electrode response is based on the enzyme-catalysed reactions:

$$\text{D-Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-Gluconic acid} + H_2O_2$$

$$H_2O_2 + 2I^- + 2H^+ \xrightarrow{\text{peroxidase}} 2H_2O + I_2$$

The highly selective iodide sensor monitors the local decrease in the iodide-ion activity at the electrode surface. The properties of these reactions were examined kinetically with flow-stream techniques and potentiometric detection. The glucose electrode so constructed and the use of flow-stream experiments with two iodide sensors provided an accurate and convenient determination of D-glucose in the absence of a number of oxidizing and reducing agents.[54] Automated methods for analysis of growth kinetics in culture media of yeast populations have included the measurement of D-glucose using the hexokinase method.[55] A manual and automated procedure for the assay of serum D-glucose using hexokinase and glucose 6-phosphate dehydrogenase was improved by replacing the light-sensitive phenazine methosulphate with 'diaphorase', thus providing a one-reagent system that could be adapted to an autoanalyser.[56] D-Glucose has been

[47] R. E. Hurst, *Carbohydrate Res.*, 1973, **30**, 143.
[48] K. J. Fidgen, *Analyt. Biochem.*, 1973, **54**, 379.
[49] N. Blumenkrantz and G. Asboe-Hensen, *Analyt. Biochem.*, 1973, **54**, 484.
[50] A. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1973, **12**, 655.
[51] D. V. Phillips and A. E. Smith, *Analyt. Biochem.*, 1973, **54**, 95.
[52] M. N. Halhoul and I. Kleinberg, *Analyt. Biochem.*, 1972, **50**, 337.
[53] F. Calhoun and H. B. Howe, *J. Chromatog. Sci.*, 1972, **10**, 639.
[54] G. Nagy, L. H. Von Storp, and G. G. Guilbault, *Analyt. Chim. Acta*, 1973, **66**, 443.
[55] J. R. Mor, A. Zimmerli, and A. Fiechter, *Analyt. Biochem.*, 1973, **52**, 614.
[56] H. J. Coburn and J. J. Carroll, *Clinical Chem.*, 1973, **19**, 127.

determined by a similar method on 20 μl samples of capillary blood, cerebrospinal fluid, or urine using the Eppendorf automatic end-point apparatus.[57] A fluorometric modification (having satisfactory specificity, accuracy, and precision) has been developed for the determination of D-glucose in whole blood without prior deproteinization.[58] Heat inactivation has been effective in removing contaminating β-glucosidase activity in enzymic reagents used for the determination of D-glucose by means of glucose oxidase.[59] A prototype apparatus utilizing glucose oxidase covalently coupled to porous glass particles has provided a reagentless analytical procedure for the estimation of D-glucose in both simple and complex biological fluids.[60] The monitor assembly involves the continuous measurement of dissolved oxygen in the sample stream. A rapid microdetermination of oxygen and D-glucose in blood has been achieved using glucose oxidase and an oxygen electrode.[61] The precision, accuracy, range of measurement, and interference by reducing substances in the estimation of D-glucose using the glucose oxidase–PERID system have been reported.[62]

A method for the assay of β-fructofuranosidase activity in a glucose oxidase–peroxidase coupled system has made use of 2-amino-2-hydroxymethylpropane-1,3-diol as an inhibitor of both invertase and contaminating glucosidases.[63] The amylase activities in sera and urine have been determined by measuring the stable colour released from Blue Starch.[64]

A new method for measuring the periodate ion is based on the principle that 4-(4′-nitrophenoxy)butane-1,2-diol is readily oxidized to produce a β-phenoxyaldehyde, which undergoes β-elimination in base to give the coloured nitrophenolate ion. This method is capable of measuring periodate concentrations as low as 10^{-8} mol l^{-1} and has been applied to the measurement of D-glucose and D-arabinitol.[65] Mercury(II), after being reduced to the elemental state, has been oxidized with sodium periodate in the presence of potassium bromide and boric acid; the alkali borate formed, which is a measure of mercury(II), was directly titrated with acid. The mercury(II) estimation has been used in the indirect determination of D-glucose, D-fructose, maltose, lactose, furfuraldehyde, gallic acid, tannic acid, formaldehyde, acetaldehyde, and benzaldehyde by reacting them with potassium mercury iodide in an alkaline medium and estimating the elemental mercury formed with sodium periodate.[66]

[57] R. Haeckel and H. Haeckel, *Z. klin. Chem. klin. Biochem.*, 1972, **10**, 453.
[58] R. Da Fonseca-Wollheim, *Z. klin. Chem. klin. Biochem.*, 1973, **11**, 24.
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[64] A. Irie, M. Hunaki, K. Bando, and K. Kawai, *Clinica Chim. Acta*, 1972, **42**, 63.
[65] D. H. Rammler, R. Bilton, R. Haugland, and C. Parkinson, *Analyt. Biochem.*, 1973, **52**, 198.
[66] R. L. Kaushik and R. Prosad, *J. Indian Chem. Soc.*, 1973, **50**, 17.

A solvolytic method has been developed for the removal of sulphate groups from acid-labile polysaccharides.[67] The degree of substitution in sodium carboxymethylcellulose has been determined by a reversed dye-partition technique using Disulphine Blue.[68]

Structural Methods

A comprehensive review of the applications of mass spectrometry to carbohydrates has been published.[69] The conformation of polysaccharides has been the subject of a review in which the following aspects are covered: random coil and conformational entropy, ordered conformations and co-operative interactions, conformational analysis, the conformations of oligosaccharides, regular and random-coil conformations of polysaccharide chains, and the interconversions between regular and disorganized conformations.[70]

The use of g.l.c.–mass spectrometry (m.s.) as an aid to structural studies continues to be widely reported. Fragmentation patterns from electron-impact m.s. have been established for the peracetylated methyl ester methyl glycoside of *N*-acetylneuraminic acid. The resulting data have allowed interpretation of the m.s. of the corresponding derivative of a new sialic acid, shown to be an 8-*O*-methyl-*N*-acetylneuraminic acid.[71] M.s. data for TMS ethers of the *O*-methyl oxime derivatives of aldoses, partially methylated aldoses, deoxyaldoses, and ketoses containing 3—7 carbon atoms have been recorded.[72] Each compound gave a distinctive spectrum indicative of the carbon-chain length and the location of substituents. Relative retention times and response factors for the TMS ethers of a number of partially methylated galactitols, L-arabinitols, and L-rhamnitols have been reported for a variety of column supports.[73] The chromatographic mobilities on a variety of stationary phases have been determined for a number of permethylated alditols and aldonates.[74] TMS derivatives of aldose diphosphates have been reported to be too unstable for g.l.c.–m.s. studies. Substituted oxime derivatives of these sugars were prepared and, although the TMS derivatives gave single peaks on g.l.c. analysis, the resolution of isomeric hexoses was poor.[75] A method for the selective degradation of polysaccharides containing uronic acid has been reported. The technique involves methylation of hydroxy- and carboxy-groups, base-

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[69] 'Biochemical Applications of Mass Spectrometry', ed. G. R. Waller, Wiley, London, 1972.
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[71] N. K. Kochetkov, O. S. Chizhov, V. I. Kadentsev, G. P. Smirnova, and I. G. Zhukova, *Carbohydrate Res.*, 1973, **27**, 5.
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[73] B. H. Freeman, A. M. Stephen, and P. van der Bijl, *J. Chromatog.*, 1972, **73**, 29.
[74] J. N. C. Whyte, *J. Chromatog.*, 1973, **87**, 163.
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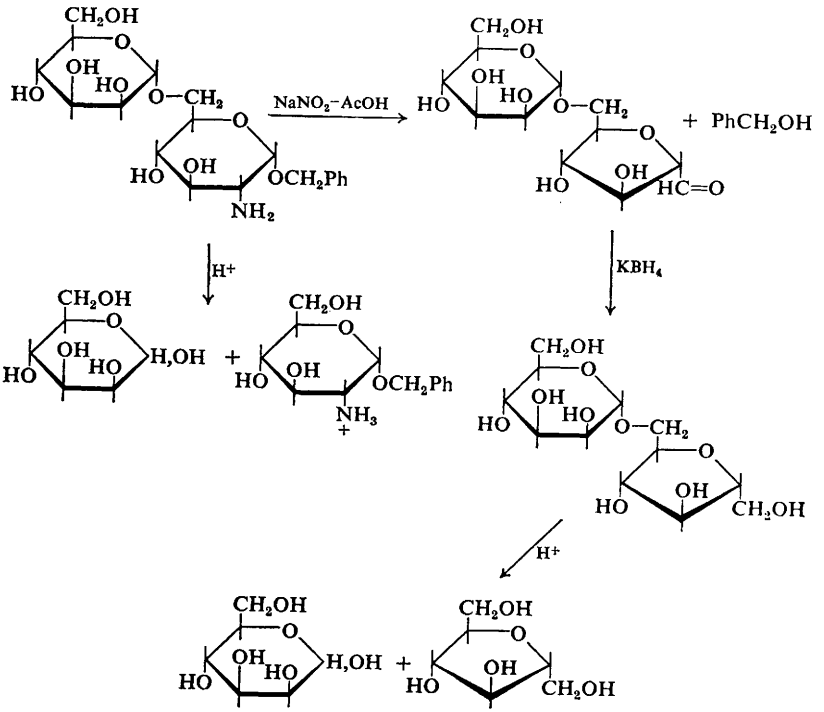
catalysed elimination, and mild acidic hydrolysis. The degraded product could then be etherified with trideuterio-methyl or -ethyl groups and, after hydrolysis, the resulting mixture of etherified sugars could be analysed as the alditol acetates by g.l.c.–m.s. Comparison of this analysis with the methylation analysis of the original polysaccharide furnished information on the nature of the sugar residues on either side of the uronic acid residue.[76] Electron-impact m.s. of the acetylated *N*-arylglycosylamine derivatives of tri-, tetra-, and penta-saccharides has allowed the determination of the molecular weight and nature and sequence of the monosaccharide residues, and in certain instances, the position of some of the interglycosidic linkages.[77] Methylated alditol acetates derived from polysaccharides have been subjected to e.s.r. and m.s. techniques to obtain information on the positions of the glycosidic linkages of the polymer.[78] Neutral disaccharides have been oxidized to the corresponding aldosyl aldonic acids which, after methylation and separation by g.l.c., have been subjected to m.s. studies.[79] The electron-impact fragmentation patterns afforded by these derivatives discriminate between (1 → 3)-, (1 → 4)-, and (1 → 6)-linked residues and differ from that postulated for (1 → 2)-intermolecular bonds. The m.s. exhibited by the oxidized products could be correlated with those obtained from reduced, permethylated pseudoaldobiouronic acids. Oxidation of methanolysates of permethylated cellulose, laminarin, and dextran with DMSO-based oxidants has been used to obtain anomeric mixtures of the corresponding dicarbonyl sugars, which were then converted into the corresponding 2,4-dinitrophenylhydrazones. High-resolution m.s. of the individual components showed distinctive patterns.[80] Syntheses of 3-*O*-methyl, 4-*O*-methyl, 3,4-di-*O*-methyl, and 3,4,6-tri-*O*-methyl ethers of methyl 2-acetamido-2-deoxy-α-D-mannopyranoside, as reference compounds in structural studies of peptidoglycans, have been reported.[81] Typical fragments, resulting from the methylation and hydrolysis of a branched D-glucan, have been separated on polyacrylamide columns.[82] The composition of mixtures of 2-*O*-methyl- and 3-*O*-methyl-xylitol acetates, which are difficult to separate by g.l.c., may be determined by either c.d. or ^{1}H n.m.r. spectroscopy.[83]

A g.l.c. method has been proposed as a means of selecting the optimum conditions for the production of glycosylalditols and residual carbohydrates after Smith degradation of polysaccharides.[84] The diagnostic

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[78] Y. N. El'Kin, B. V. Rozynov, and A. K. Izuzenko, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 710.
[79] J. N. C. Whyte, *Canad. J. Chem.*, 1973, **51**, 3197.
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[81] Nasir-ud-Din and R. W. Jeanloz, *Carbohydrate Res.*, 1973, **28**, 243.
[82] E. Grellert and C. E. Ballou, *Carbohydrate Res.*, 1973, **30**, 218.
[83] G. G. S. Dutton and N. A. Funnell, *Canad. J. Chem.*, 1973, **51**, 3190.
[84] G. G. S. Dutton and K. B. Gibney, *Carbohydrate Res.*, 1972, **25**, 99.

value has been considered for the degradation products arising when the Smith-degradation procedure is modified by introduction of a methylation stage after sodium borohydride reduction of periodate-oxidized polysaccharides.[85] The bond types occurring in two clinical dextrans were examined by conventional periodate oxidation and methylation analysis and by the modified procedure. Mono-*O*-methylhexoses, di-*O*-methylhexoses (2,4-; 2,6-; 3,6-; and 4,6-) containing no vicinal methoxy-groups, and 2,4,6-tri-*O*-methylhexoses were obtained, depending on the linkage pattern of the periodate-resistant hexose. Some new methyl ethers of the tetritols, *viz.* 1-*O*-methyl-D-erythritol, 1-*O*-methyl-L-threitol, 1,4-di-*O*-methylerythritol, and 1,4-di-*O*-methyl-L-threitol, have been synthesized as reference compounds for use with this method.[86] A simple method for preparing the acetylated, derived aldononitriles and alditols, and their use in quantitative g.l.c. of typical Smith-degradation products of glucans have been reported.[87] The chain lengths of various glucans were estimated using this technique. The consumption of periodate by fractions of ovalbumin containing 2-acetamido-2-deoxy-β-D-glucopyranosyl residues was found to be less than the theoretical value when measured spectrophotometrically.[88] A significant part of the discrepancy is explained by the absorbance of oxidized 2-acetamido-2-deoxyhexosyl residues at the wavelength of the assay. A semi-micro determination of sugar configuration (D or L) can been conveniently carried out on alditol acetates or their methylated derivatives by c.d. at 213 nm, where the acetoxy-group acts as a chromophore.[89] A procedure has been described for the deamination of glycosides and acetates of 2-amino-2-deoxy-D-glucose with nitrous acid and for reduction of the product to 2,5-anhydro-D-mannitol.[90] The results have provided a model for a non-hydrolytic depolymerization technique for the characterization of glycosaminoglycans. Studies on the selective cleavage of glycosidic linkages have been reported using benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside as a model compound.[91] De-*N*-acetylation of this compound with either 2.5 M-NaOH or with hydrazine in the presence of hydrazine sulphate proceeded quantitatively and was not accompanied by destruction of the bioside. The mannosyl glycosidic linkage can then be selectively hydrolysed with acid, whereas the 2-amino-2-deoxyhexosyl linkage can be selectively cleaved with nitrous acid to 2,5-anhydro-6-*O*-α-D-mannopyranosyl-D-mannose and benzyl alcohol (Scheme 1). The de-*N*-acetylation of benzyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside was similarly

[85] P. Nánási and A. Lipták, *Carbohydrate Res.*, 1973, **29**, 193.
[86] P. Nánási and A. Lipták, *Carbohydrate Res.*, 1973, **29**, 201.
[87] J. K. Baird, M. J. Holroyde, and D. C. Ellwood, *Carbohydrate Res.*, 1973, **27**, 464.
[88] J. Eltin, C. C. Huang, and R. Montgomery, *Carbohydrate Res.*, 1973, **28**, 387.
[89] G. M. Bebault, J. M. Berry, Y. M. Choy, G. G. S. Dutton, N. Funnell, L. D. Hayward, and A. M. Stephen, *Canad. J. Chem.*, 1973, **51**, 324.
[90] D. Horton and K. D. Philips, *Carbohydrate Res.*, 1973, **30**, 367.
[91] B. A. Dmitriev, Y. A. Knirel, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **30**, 45.



Scheme 1

achieved, after which the galactosyl glycosidic bond was selectively cleaved by acidic hydrolysis, whereas nitrous acid released 2,5-anhydro-3-*O*-β-D-galactopyranosyl-D-mannose and benzyl alcohol.[92] By use of peptidases and a glucosylamidase from *Limnaea stagnalis*, a method has been devised for establishing the nature of the carbohydrate–peptide bond in glycoproteins.[93]

Curie-point pyrolysis–g.l.c. has been used to discriminate between polysaccharides differing only in structural aspects, as evidenced from preliminary work on structurally different glucans.[94] A technique has been described for coating small quantities of insoluble samples to the ferromagnetic filaments used in the Curie-point pyrolytic procedure. The cell-wall polysaccharides from a streptococcal strain and its mutant strain lacking a polysaccharide antigen have been differentiated by this method.[95]

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[95] J. H. J. Huis In't Veld, H. L. C. Meuzelaar, and A. Tom, *Appl. Microbiol.*, 1973, **26**, 92.

Absorptions near 940 and 970 cm^{-1} in their i.r. spectra have been reported to be of value in identifying the main glycosidic linkages of mannans.[96] The presence of acetate and pyruvate groups in polysaccharides has been demonstrated and estimated quantitatively by measuring the ^1H n.m.r. spectrum of the polysaccharide in deuterium oxide at 95 °C. The spectra also permitted an assessment to be made of the number of α- and β-linkages in the repeat unit of the structure.[97] An evaluation of the potential of longitudinal nuclear relaxation times as a probe for structural assignments of carbohydrate derivatives has been made for a number of oligosaccharides.[98] For the reducing end-group, differences in relaxation times were noted for axially and equatorially oriented protons. The relaxation time of the anomeric proton of the non-reducing end-group also showed a configurational dependence and provided a basis for assigning the individual resonances to the anomeric protons of oligosaccharides. The ^{13}C n.m.r. spectra of some mannose-containing disaccharides have been measured and compared with those of related mono- and di-saccharides. Measurements before and after mutarotational equilibrium had been attained facilitated the assignment of signals to α- and β-anomers.[99]

The conversion of xylitol into D-xylulose by sorbitol dehydrogenase has been used as the basis for determining the number-average degree of polymerization ($\overline{DP}_n$) of a number of xylo-oligosaccharides and xylans. The method enables the proportion of xylitol to reducing sugar present in the reduced ($NaBH_4$) carbohydrates to be determined after hydrolysis with acid.[100] Reduction with sodium borotritide of a series of maltose oligosaccharides has yielded products whose residual radioactivity has a linear dependence on the degree of polymerization. The procedure was extended to measure the number-average molecular weights of hydrolysed amyloses.[101] A short-column sedimentation method has been applied to the determination of the molecular weights of oligosaccharides. Theoretical considerations regarding the basic equilibrium equations were made for ideal monodispersed, non-ideal monodispersed, and ideal polydispersed solutions, and two methods for the computation of molecular weights were proposed.[102]

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3
Plant and Algal Polysaccharides

BY R. J. STURGEON

Introduction

A number of reviews and authoritative reports have been published on plant and algal polysaccharides under the following titles: Polysaccharide Conformation, Polysaccharide Methodology, and Plant Polysaccharides,[1] The Industrial Uses of Carbohydrates – Present and Future,[2] ^{1}H N.M.R. Spectroscopy of Polysaccharides,[3] Polysaccharide Gels – a Molecular View,[4] Pectic Substances and Pectinolytic Enzymes,[5] The Biosynthesis of Complex Polysaccharides,[6] and The Fine Structure of Starch and its Relationship to the Organization of Starch Granules.[7]

Starch

A procedure has been described for simultaneous optimization of bond lengths and angles for the D-glucose residues and the chain conformation of the β-amylose helix while performing a packing analysis for the chains in a crystalline assay.[8] The most probable packing of the amylose chains in the cell is considered to be antiparallel with the chains in a left-handed six-fold helical conformation and with the O-6 atoms in the *gt* rotational position. The effects of changes in ring geometry on computer models of amylose have been reported.[9] By use of D-glucopyranose residues having different geometries, it was possible to build models of V-amylose helices having six, seven, and eight residues per turn, single and double helical β-amyloses, and KBr–amylose. Because no single residue could satisfactorily model all of the known polymorphs of amylose, it was suggested that structural determinations utilizing a rigid-residue approximation should make use of the full range of known residue geometries.

[1] G. O. Aspinall, in 'Carbohydrates', ed. G. O. Aspinall, in 'MTP International Review of Science, Organic Chemistry Series One', Butterworths. London, 1973, Vol. 7, p. 275.
[2] M. Stacey, *Chem. and Ind.*, 1973, 222.
[3] B. Coxon, *Adv. Carbohydrate Chem. Biochem.*, 1972, **27**, 39.
[4] D. A. Rees, *Chem. and Ind.*, 1972, 630.
[5] W. M. Fogarty and O. P. Ward, *Process Biochem.*, 1972, 13.
[6] 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Academic Press, New York and London, 1972, Vol. 28B.
[7] A. D. French, *J. Jap. Soc. Starch Sci.*, 1972, **19**, 8.
[8] P. Zugenmaier and A. Sarko, *Biopolymers*, 1973, **12**, 435.
[9] D. French and V. G. Murphy, *Carbohydrate Res.*, 1973, **27**, 391.

The failure of native B-starch to collapse during drying has been taken as an indication that water is not intercalated between turns of the helix or otherwise required to maintain the geometry and packing arrangement of the molecular chains.[10] When amylodextrin was dried with a non-crystalline starch syrup, small molecules of the syrup were able to penetrate the interstices between crystallites and so prevent strain building up during the drying process. A model for B-starch was proposed that employed intertwined double helices. Adsorption–desorption isotherms for water vapour on starch have been obtained by use of a quartz-fibre spring technique.[11] The specific surface area of starch has been calculated by two methods. The Raman spectra of V_a-, V_h-, and B-forms of amylose have been recorded and interpreted in terms of a proposed mechanism for V → B conversion involving extension of the helix and changes in intramolecular hydrogen bonding.[12] Spectra of amylose dissolved in aqueous salt solution and DMSO have also been recorded; the results indicated that amylose does not adopt the V-conformation in DMSO. Data from light-scattering, sedimentation equilibrium, sedimentation velocity, and viscosity measurements have been interpreted using the Eizner–Ptitsyn equation for worm-like chains. Except in the molecular-weight region of 1×10^6, the observed values fitted this equation and the disagreement in the high molecular-weight region was tentatively attributed to the excluded-volume effect.[13] In the distribution of tritium into D-glucose and starch after labelling by tritium-atom bombardment, 41% of the label was incorporated into the amylose–butyl alcohol complex, with almost no label on C-3 and twice the expected label on C-2 of the D-glucose residues.[14] The use of liquid ammonia as a non-aqueous solvent for starch has been reviewed.[15]

Contrary to previous views, it has been reported that the intrinsic viscosity of solutions of potato amylose decreases significantly upon complex formation with iodine.[16] These findings have been interpreted in terms of an amylose model characterized by loose, extended, helical regions interrupted by short disordered regions; the decrease of intrinsic viscosity was attributed to shortening of the linear dimension of the polymer chain. The change of conformation is apparently caused by a contraction of the loose helical regions of the amylose macromolecule owing to the entrapment of iodine inside the helical cavities. The rate of iodine absorption by granular starch is reported to be controlled by the intraparticle diffusion of iodine.[17] Unit-cell dimensions of amylose–fatty acid complexes have been calculated for both wet and dry states.[18] Both the six- and seven-

[10] K. Kainuma and D. French, *Biopolymers*, 1972, **11**, 2241.
[11] B. Das, R. K. Sethi, and S. L. Chopra, *Israel J. Chem.*, 1972, **10**, 963.
[12] J. J. Cael, J. L. Koenig, and J. Blackwell, *Carbohydrate Res.*, 1973, **29**, 123.
[13] M. Fujii, K. Honda, and H. Fujita, *Biopolymers*, 1973, **12**, 1177.
[14] T. Liang, P. Nordin, and H. Moser, *Carbohydrate Res.*, 1973, **27**, 437.
[15] C. J. Moye, *Adv. Carbohydrate Chem. Biochem.*, 1972, **27**, 91.
[16] M. B. Senior and F. Hamori, *Biopolymers*, 1973, **12**, 65.
[17] Y. Y. Lee and G. T. Tsao, *Stärke*, 1973, **25**, 166.
[18] K. Takeo, A. Tokumura, and T. Kuge, *Stärke*, 1973, **25**, 357.

helical conformations of amylose were found in these complexes, with the conformation depending on the linear chain length of the fatty-acid molecules. In thermodynamic compatability studies of gelatin with glucans in aqueous media, it was established that all the systems investigated, *viz.* water–gelatin–D-glucan (amylopectin, dextran, or glycogen), are thermodynamically unstable under isoionic conditions at sufficiently high concentrations of polymers.[19] However, when the pH was shifted away from the pI of gelatin, and also when the ionic strength was increased sufficiently, the systems underwent a reversible phase transition from a two-phase to a single-phase state. This suggested that the thermodynamic incompatibility of gelatin with D-glucans in isoionic conditions is determined by self-association of gelatin macro-ions owing to interaction of charge fluctuations.

An enzymic method has allowed the direct examination of the constitutive chains of starches, thereby providing information on the fine structure of these molecules.[20] After treatment with pullulanase, the debranched chains were separated by gel-permeation chromatography and their linearity was checked by treatment with β-amylase, whilst the inner chains of the amylopectin fraction were studied after debranching of the β-limit dextrins. By use of this technique it was shown that the amylopectin from amylomaize has longer inner chains than those of waxy maize amylopectin. From a study of the swelling properties and solubility of a waxy Compana starch, it has been suggested that this variety of barley contains a unique amylopectin containing unusually long branches or natural cross-links.[21] The solubility of starches in DMSO has been reported to be characteristic of the species, variety, and maturity of the plant.[22] Chemical analysis of a fraction insoluble in this solvent indicated that it is a highly branched amylopectin. Damaged starch in flour has been estimated by polarimetric determination of the starch in calcium chloride solution and from the observation that damaged starch is more readily digested by α-amylase than undamaged starch.[23] An amylose to amylopectin ratio of 8 : 2 has been reported to be the limit of detectability of these polymers by column chromatographic methods.[24] Ethanol-induced adsorption of amylose on a cellulose column has been used for the quantitative separation of amylose and amylopectin from starch.[25] The amylose was recovered by gradient elution, when the elution profile revealed heterogeneity of the native amylose. Amylose samples have been shown to be heterogeneous by paper chromatography.[26] The acetyl values of starches have been measured by

[19] V. Y. Grinberg and V. B. Tolstoguzov, *Carbohydrate Res.*, 1972, **25**, 313.
[20] C. Mercier, *Stärke*, 1973, **25**, 78.
[21] K. J. Goering, R. Eslick, and B. W. De Haas, *Cereal Chem.*, 1973, **50**, 322.
[22] R. H. Kurtzman, F. T. Jones, and G. F. Bailey, *Cereal Chem.*, 1973, **50**, 312.
[23] B. Y. Chiang, G. D. Miller, and J. A. Johnson, *Cereal Chem.*, 1973, **50**, 44.
[24] J. Seidemann, *Stärke*, 1973, **25**, 211.
[25] N. B. Patil and N. R. Kale, *J. Chromatog.*, 1973, **84**, 75.
[26] J. Seidemann, *Makromol. Chem.*, 1973, **167**, 79.

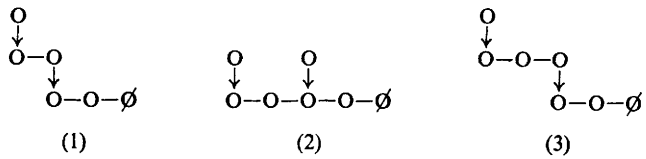
i.r. absorption spectroscopy; the values reported are in agreement with those obtained by other methods.[27] The influence of radiation on such properties of starch as humidity, glucosidic bonds, and conditions of storage has been examined.[28] Proposals have been made regarding the kind of sugars formed after such treatment and on the role of water in the radiolytic process.

The kinetics and products formed on oxidation of starch with bromine water have been reported.[29] The i.r.s pectra of the products from starch indicated the presence of carboxylic and aldehydic groups, whereas carboxylic and carbonyl groups were detected in the products from amylose, the amounts of which varied with the pH at which the oxidation had been conducted. The hydrolysis products of oxidized amylose included D-glucurono-6,3-lactone and D-glucuronic acid. An examination of the oxidation of starch with sodium hypochlorite has shown that hydroxy-groups take an active part in the oxidation, the rate of which decreased when the hydroxy-groups on C-2 and C-3 were substituted.[30] An investigation of the oxidation of starch with chlorine in deuterium oxide suggested that the formation of protons in an acidic medium, and the repulsion between anions in an alkaline medium, decreased the oxidation rate. When starch was submitted to hydrolysis under high temperatures and pressures at neutral pH values, a series of monohydric alcohols, carbonyl compounds, D-glucose, and small amounts of mannose, xylose, and arabinose were detected.[31]

The molecular mechanism of amylolytic enzymes on amylose has been investigated by a variety of methods using pancreatic and bacterial α-amylases, and amyloglucosidase.[32] It was concluded that the filling between the substrate and the enzyme is induced by the hydroxy-groups at C-3 of the substrate. In the course of the filling, hydrogen bonds arise between these hydroxy-groups and those of the enzyme molecules possessing the highest affinity. A new theory was proposed to explain the high catalytic effectiveness of these enzymes. The rate of hydrolysis of amylopectin by three different limit dextranase preparations was found to be very much less than that of amylopectin β-limit dextrin under similar conditions.[33] The small affinity for amylopectin is considered to be of little importance *in vivo*, since only α-amylases of the commonly occurring starch-metabolizing enzymes are able to attack native starch granules in plant tissues. When starch granules of various botanical origins were subjected to hydrolysis by porcine pancreatic juice, all the granules were found to be damaged. However, the kind of attack was peculiar to each species, as

[27] V. Prey, H. Schindlbauer, and E. Maday, *Stärke*, 1973, **25**, 73.
[28] G. Berger, J. P. Agnel, and L. Saint-Lebe, *Stärke*, 1973, **25**, 203.
[29] I. Ziderman and J. Bel-Ayche, *Carbohydrate Res.*, 1973, **27**, 341.
[30] K. F. Patel, H. U. Mehta, and H. C. Srivastava, *Stärke*, 1973, **25**, 266.
[31] K. Lorenz and J. A. Johnson, *Cereal Chem.*, 1972, **49**, 616.
[32] J. Holló, E. László, and Á. Hoschke, *Stärke*, 1973, **25**, 1.
[33] G. Dunn, D. G. Hardie, and D. J. Manners, *Biochem. J.*, 1973, **133**, 413.

evidenced by transmission- and scanning-electron microscope studies.[34] In contrast to the characteristically slow hydrolysis of intact potato- and maize-starch grains by glucoamylase and α-amylase, a rapid and complete hydrolysis of leaf-starch grains has been reported.[35] It has been postulated that leaf-starch grains may differ from storage-starch grains in a way that might be relevant to their rapid, diurnal breakdown in the leaf. A strain of *Bacillus licheniformis* has been shown to produce an extracellular α-amylase having unusual characteristics; the predominant product of its action on amylose and amylopectin during all stages of hydrolysis was maltopentaose.[36] The smallest α-dextrin produced from amylopectin by a *B. subtilis* α-amylase has been shown by a combination of chemical and enzymic degradations to be 6^2-α-maltosylmaltotriose.[37] Branched dextrins consisting of six D-glucose units have been isolated after treatment of amylopectin with an α-amylase.[38] The positions of the α-1,6-linkages differ from each other but, by use of α-amylase, β-amylase, glucoamylase, and pullulanase, it was demonstrated that the singly-branched hexaose dextrins were 6^2-α-maltotriosylmaltotriose, 6^2-α-maltosylmaltotetraose, and 6^3-α-maltosylmaltotetraose. The branched dextrins produced by the saccharifying α-amylase of *B. subtilis* on the β-limit dextrin of waxy rice starch have been isolated.[38a] Analysis of the products of this digestion by a range of starch-degrading enzymes revealed the presence of three doubly branched dextrins, whose structures were assigned as 6^3-α-(6^2-α-glucosylmaltosyl)maltotriose, 6^3-α-(6^5-diglucosyl)maltopentaose, and 6^3-α-(6^3-α-glucosylmaltotriosyl)maltotriose (1)—(3).



(1) (2) (3)

O→ = α-D-glucose (1→6)-
O— = α-D-glucose (1→4)-
Ø = reducing D-glucose unit.

The kernels of mango seeds (*Mangifera indica*) contain starch made up of 65% amylose and 35% amylopectin.[39] A glycogen-type polysaccharide has been characterized from the plastids of the higher green plant *Cecropia peltata*; enzymic studies showed that the polysaccharide is similar to phytoglycogen.[40] The amylose molecules of sweet potato starch at an early

[34] D. Gallant, A. Derrien, A. Aumaitre, and A. Guilbot, *Stärke*, 1973, **25**, 56.
[35] R. W. Bailey and J. C. Macrae, *F.E.B.S. Letters*, 1973, **31**, 203.
[36] N. Saito, *Arch. Biochem. Biophys.*, 1973, **155**, 290.
[37] D. French, E. E. Smith, and W. J. Whelan, *Carbohydrate Res.*, 1972, **22**, 123.
[38] K. Umeki and T. Yamamoto, *J. Biochem. (Japan)*, 1972, **72**, 101.
[38a] K. Umeki and T. Yamamoto, *J. Biochem. (Japan)*, 1972, **72**, 1219.
[39] E. S. Amin and M. M. El-Sayed, *Carbohydrate Res.*, 1973, **27**, 39.
[40] J. J. Marshall and F. R. Rickson, *Carbohydrate Res.*, 1973, **28**, 31.

stage of development have been shown to be relatively small, to be sensitive to the action of dilute alkali, and to have a much longer chain length than the corresponding molecules isolated from mature tubers.[41] Changes in the carbohydrate constituents of the yam tuber, *Dioscorea rotundata*, during growth have also been measured.[42] Starch, which accounted for 84% of the carbohydrates after six months of growth, had dropped to 64% after eight months. Changes in the formation of starch during the development of wheat and barley kernels have been reported.[43]

The following aspects of the biosynthesis of starch have been reported: a primerless form of potato phosphorylase with a possible involvement in starch biosynthesis, studies on particulate transglucosylases from potato tubers, the regulatory mechanisms in the biosyntheses of plant starch and bacterial glycogen, and the synthesis of an α-(1 → 4)-glucan by ADP-glucose:α-1,4-glucan 4-glucosyltransferases in the absence of primer.[44] Evidence has been presented for the existence of two types of starch synthetase in varieties of maize.[45] It was proposed that amylose is synthesized by a synthetase *per se*, whereas amylopectin is synthesized by a synthetase-branching enzyme complex. The ratio of amylose : amylopectin formed is a function of the ratio of the two forms of synthetase present. A particulate fraction of protoplastids from potato tubers has been reported to transfer D-glucose from UDP-D-glucose to a trichloroacetic-acid-insoluble product having the properties of a glucoprotein, and which can act as an acceptor in the synthesis of an α-(1 → 4)-glucan from ADP-D-glucose or D-glucose 1-phosphate.[46] Changes in the molecular components of barley starch from related genotypes of barley differing in amylose content have been investigated during the deposition of reserve starch in the plants.[47] Striking similarities in the changes in amylose contents, and in the fine structure of the component amylose and amylopectin fractions, were observed over this period despite large differences in the types of starch. However, later work showed that the patterns by which the granules are laid down differ markedly.[48] With the exception of phosphoenol pyruvate carboxykinase, all the enzymes necessary for the synthesis of sucrose and polysaccharides from metabolites of the citric-acid cycle have been found in the phloem exudate of *Cucurbita pepo*.[49] The polysaccharide synthetase was found to exhibit higher activity with glycogen than with starch and it was stimulated by D-glucose 6-phosphate. Biochemical changes taking place during germination of the coconut, *Cocos*

[41] S. Fujimoto, T. Nagahama, and M. Kanie, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 577.
[42] A. O. Ketiku and V. A. Oyenuga, *J. Sci. Food Agric.*, 1973, **24**, 367.
[43] J. Cerning and A. Guilbot, *Cereal Chem.*, 1973, **50**, 220.
[44] 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[45] S. Schiefer, E. Y. C. Lee, and W. J. Whelan, *F.E.B.S. Letters*, 1973, **30**, 129.
[46] N. Lavintman and C. E. Cardini, *F.E.B.S. Letters*, 1973, **29**, 43.
[47] W. Banks, C. T. Greenwood, and D. D. Muir, *Stärke*, 1973, **25**, 153.
[48] W. Banks, C. T. Greenwood, and D. D. Muir, *Stärke*, 1973, **25**, 225.
[49] J. Lehmann, *Planta*, 1973, **114**, 51.

nucifera, have been examined.[50] The total starch content of the haustorium increased linearly with maturation, whereas the concentrations of reducing and soluble sugars rose very rapidly and remained at a steady state thereafter. The results suggested that the embyro metabolizes the starch stored in the haustorium during germination, probably to be utilized during unfavourable conditions.

Cellulose

Examination of powder *X*-ray diffractograms of native and hydrolysed cellulosic materials from various sources has revealed the presence of materials having a higher degree of molecular order than the ramie hydrolysate (the conventional crystalline standard for the cell).[51] By adopting these materials as new crystalline standards, a critical reappraisal has been made of the validity of the two-phase (fringed-micelle) hypothesis and it was concluded that the lattice structure of cotton and related celluloses of plant and bacterial origin is liquid-like or paracrystalline. Successful attempts have been made to crystallize cellulose II by hydrolysis of cellulose acetate.[52] The polysaccharide could be crystallized in a variety of structural forms if the strong intermolecular hydrogen-bonding, which under normal circumstances dominates the flexibility of the backbone, is avoided by using the appropriate experimental conditions. *X*-Ray diffraction data of fresh, undried cotton fibres removed from cotton bolls at various stages of fibre growth, have been presented.[53] A considerable extent of cellulose crystallization and the presence of water was noted. Growth of the cell wall resulted in an increase in crystallization and a decrease of water content in the undried fibres. A study of the polymolecularity of irradiated pulps from spruce, reed, and straw showed that destruction of cellulose occurs.[54] At a secondary level, the action of 50 Mrad doses of radiation did not influence the crystallinity of cellulose I, whereas higher doses resulted in a slight increase in the content of crystalline material for cotton and spruce pulps. A decrease in crystallinity was induced in cellulose II. A dependence between the stability of hydrogen-bonding of cellulose molecules and the rate of ethanolysis has been reported, together with a method for estimating the content and stability of these bonds in loose and dense sections of cellulose samples.[55] A systematic approach to stereochemical aspects of the conformational analysis of cellulose, polyguluronic

[50] K. Balasubramaniam, T. M. S. Atukorala, S. Wijesundera, A. A. Hoover, and M. A. T. De Silva, *Ann. Botany*, 1973, **37**, 439.
[51] A. K. Kulshreshtha and N. E. Dweltz, *J. Polymer Sci., Polymer Phys. Edn.*, 1973, **11**, 487.
[52] R. Ramesh, C. K. Patel, and R. D. Patel, *Makromol. Chem.*, 1973, **171**, 179.
[53] A. K. Kulshreshtha, K. F. Patel, A. R. Patel, M. M. Patel, and N. T. Baddi, *Cellulose Chem. Technol.*, 1973, **7**, 343.
[54] C. Simionescu, R. Butnaru, and G. Rozmarin, *Cellulose Chem. Technol.*, 1973, **7**, 153.
[55] V. I. Sharkov, V. P. Levanova, and A. K. Bolotova, *Cellulose Chem. Technol.*, 1972, **6**, 263.

acid, mannan, and polymannuronic acid has been undertaken. A more-exact treatment of the main side-groups has been given, and differences from the approximate method previously used were found. A comparison of the flexibility of the four polysaccharides has indicated that the polyuronic acids are more flexible than the corresponding glycans and that the mannans are more flexible than the glycans.[56] The viscosity–DP relation of narrow fractions of cellulose of low DP has been determined in cadoxene in order to obtain estimates of the axial ratios of the polysaccharide.[57] The cellulose–cadoxene complex was shown to conform to the disk-like oblate model at a DP of 31, but this was followed by a gradual departure from the oblate to the prolate model. Calculations based on the potential energy associated with the rotational angle in cellulose showed that cellulose is incapable of random coiling at less than DP 2×10^3, indicative of the flexibility of the polysaccharide.

A study of the cellulose–dye adsorption process by the monolayer method has indicated the existence of weak complexing, probably of an acid–base type, between cellulose and anionic groups of the dyes.[58] The partitioning behaviour of a homologous series of polar and non-polar compounds of low molecular weight (*e.g.* cellodextrins, cellodextrin acetates, polyethylene oxides, polyhydric alcohols, and methyl oligo(*p*-phenylenes) was studied on cellulose gels and derivative forms thereof such as acetates, methyl ethers, and TMS ethers.[59] An inverse relationship was observed between the degree of interaction of a given compound with the cellulose gel and that with a particular derivative. Thus, the most polar compound, D-mannitol, interacted strongly with the cellulose gel and weakly with the methylated gel, whereas the least polar compound, polyethylene oxide, interacted weakly with the cellulose gel and strongly with the methylated gel. From a study of the selectivities of highly cross-linked cellulose gels in the separation of anions and cations, the nature of the gel matrix, as distinct from the identity of the fixed charges, was shown to be of importance in determining the order of elution.[60] The binding of calcium ions to such polyglucose matrices as cellulosic dialysis membranes and dextrans was in amounts sufficient to influence markedly the rate of dialysis of the ions.[61] In applications where such interaction is critical (*e.g.* in binding studies by flow dialysis or ultrafiltration), the binding was prevented by either acetylation of the membrane or use of a high concentration of salt.

The comparative reactivity of cellulose and mixed polysaccharides containing D-gluco- and altro-pyranose units in acetylation, nitration, etherification, and hydrolysis reactions has been investigated.[62] The influence of

[56] E. D. T. Atkins, E. D. A. Hopper, and D. H. Isaac, *Carbohydrate Res.*, 1973, **27**, 29.
[57] H. A. Swenson, *Tappi*, 1973, **56**, 106.
[58] V. G. Agnihotri and C. H. Giles, *J.C.S. Perkin II*, 1972, 2241.
[59] K. Chitumbo and W. Brown, *J. Chromatog.*, 1973, **80**, 187.
[60] W. Brown and K. Chitumbo, *J. Chromatog.*, 1971, **63**, 478.
[61] K. C. Reed, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 1136.
[62] L. S. Galbraich, I. L. Voytenko, and Z. A. Rogovin, *Cellulose Chem. Technol.*, 1972, **6**, 627.

the changes in the configuration of the secondary hydroxy-groups and in the conformation of the elementary unit on the rates of acetylation and methylation has been measured. On the basis of such physicochemical characteristics as sorption and heat of solvation, the relationship between the conformation of the elementary unit and the structure of the polysaccharide was found. Only hydrogen, carbon monoxide, and carbon dioxide were evolved on photolysis of hydrocellulose by light of 253.7 nm.[63] Pyrolysis of cellulose under vacuum produced varying amounts of 1,6-anhydro-β-D-glucopyranose, 1,6-anhydro-β-D-glucofuranose, α- and β-D-glucopyranoses, 3-deoxy-D-*erythro*-hexulose, oligo- and poly-saccharides, and some dehydration products.[64] The polysaccharide fraction had no end-group, was randomly linked, contained some furanoid rings, and was very similar to the polysaccharide-condensation product of 1,6-anhydro-β-D-glucopyranose. These results are consistent with the occurrence of a series of inter- and intra-molecular transglycosylation and dehydration and rehydration reactions. Chemical factors have been shown to influence the thermal destruction of cellulose.[65] The presence of carboxylic acid and carbonyl groups accelerated the thermal destruction, especially in the initial stages of treatment; the distribution of these functional groups also had an effect. Differential thermal analyses have been carried out on samples of brominated wood with varying contents of bromine, and on a sample of isolated bromolignin.[66] The resultant thermograms were interpreted in terms of the pyrolytic processes occurring in both the cellulose and lignin components of wood, and showed that changes in the processes result from increased concentrations of bromine. Samples of wood from *Arundo donax* and *Phragmites communis*, after irradiation with ^{60}Co, had constant cellulose : lignin ratios, although some chemical modifications in carbohydrates of low DP were shown to influence the proportions of substances extracted.[67] The influence of the biological age of spruce wood in a sulphite-extraction procedure was shown in differences in chemical and anatomical structures along the diameter of the stem, which were revealed by differences in the viscosity and the yield of cellulose.[68] The yield of cellulose depended on the content of Krüschner–Hoffer cellulose in the age-fraction of the wood and, although the viscosity depended directly on the yield, it was independent of the permanganate number of the cellulose. When the variation with temperature of the specific volume of some hydrocelluloses in toluene was investigated and the DPs determined during the course of acidic hydrolysis of the polysaccharide, the DP was found to decrease rapidly with the progress of hydrolysis but

[63] A. Bos and A. S. Buchanan, *J. Polymer Sci., Polymer Chem. Edn.*, 1973, **11**, 833.
[64] F. Shafizadeh and Y. L. Fu, *Carbohydrate Res.*, 1973, **29**, 113.
[65] B. Philipp, J. Baudisch, and W. Stohr, *Cellulose Chem. Technol.*, 1972, **6**, 379.
[66] A. Basch, B. Hirschmann, and M. Lewin, *Cellulose Chem. Technol.*, 1973, **7**, 255.
[67] G. Rozmarin and R. Butnaru, *Cellulose Chem. Technol.*, 1972, **6**, 393.
[68] Y. N. Nepenin, G. A. Pazukhina, V. A. Diomin, and T. A. Rybakova, *Cellulose Chem. Technol.*, 1973, **7**, 235.

soon to reach a constant value.[69] Plots of the specific volumes in toluene and in water against temperature showed that the transition exhibited by cellulose fibres at about 25 °C was retained only by residues in the initial stages of hydrolysis and gradually became less marked with the progress of hydrolysis, until it vanished when the levelling-off DP was reached. The heat of wetting of some hydrocelluloses in water was shown to decrease sharply in the initial stages of hydrolysis, after which a constant value was attained.[70] The rates of hydrolysis of cellulose and the rates of formation of cellulose–formaldehyde were shown to be related to the acidity functions (H_0) of the solutions.[71]

The effect of delignification on the *in vitro* digestion of the cellulose and hemicellulose of sugar-cane bagasse by rumen was to allow increased levels of breakdown of these polysaccharides.[72] The portion of spear grass, *Heteropogon contortus*, which resisted digestion by bovine rumen showed lower contents of cellulose and higher contents of hemicellulose than fresh spear grass.[73] The resistance of these polysaccharides to complete digestion by the rumen appeared to be due to physical protection by the lignin. As much as 65% of cellulose could be digested by cell-free enzymes from *Ruminococcus albus* and most of the enzymes were absorbed on to the cellulose.[73a] Absorption and desorption characteristics, as well as partial inhibition by oxygen, indicated that multiple enzymes are involved.

A reversed dye-partition technique using Disulphine Blue has been used to determine the degree of substitution of sodium carboxymethylcellulose.[74] Oxidation of the products of methanolysis of permethylated cellulose has yielded anomeric mixtures of the corresponding dicarbonyl sugars.[75] High-resolution mass spectra of the derived 2,4-dinitrophenylhydrazones showed distinctive characteristics.

The cell walls of four-week-old *Phaseolus aureus* seedlings were shown to contain 60% α-cellulose, compared with half this value in four-day-old seedlings.[76] A study was made of the incorporation of D-[^{14}C]glucose into the cell-wall polysaccharides of the bean stem. It was found that the hemicellulosic fraction showed a higher rate of incorporation of the label than the α-cellulose in the early stages of growth but, with increasing age of the plant, radioactivity was transferred from the hemicellulosic fraction to the α-cellulose, suggesting a turnover of polysaccharides in the growing cell wall. A low-molecular-weight microsome-bound fraction increased with time when excised etiolated cotton hypocotyls were exposed to air.[77]

[69] K. Aziz and H. G. Shinouda, *Cellulose Chem. Technol.*, 1973, **7**, 465.
[70] K. Aziz and H. G. Shinouda, *Cellulose Chem. Technol.*, 1973, **7**, 479.
[71] A. Mohn-Wehner, H. K. Rouette, and H. Zollinger, *Helv. Chim. Acta*, 1973, **56**, 323.
[72] R. F. H. Dekker and G. N. Richards, *J. Sci. Food Agric.*, 1973, **24**, 375.
[73] R. J. Beveridge and G. N. Richards, *Carbohydrate Res.*, 1973, **28**, 39.
[73a] W. R. Smith, I. Yu, and R. E. Hungate, *J. Bacteriol.*, 1973, **114**, 729.
[74] S. Mukhopadhyay, B. C. Mitra, and S. R. Palit, *Analyt. Chem.*, 1973, **45**, 1775.
[75] N. Kashimura, K. Yoshida, and K. Onodera, *Carbohydrate Res.*, 1972, **25**, 264.
[76] G. Franz, *Planta*, 1972, **102**, 334.
[77] D. G. Rusness and D. S. Frear, *J. Expt. Botany*, 1973, **24**, 272.

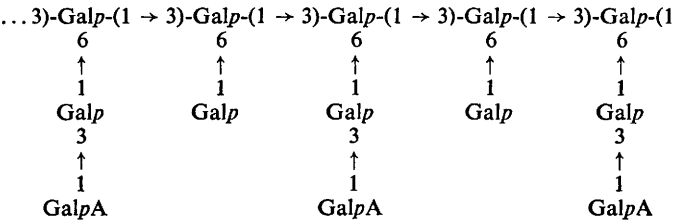
The activities of two microsomal enzyme systems involved in β-glucan biosynthesis were altered as a result of the formation of this fraction, and up to a five-fold increase in the ratio of non-cellulosic : cellulosic D-glucosyl linkages synthesized from UDP-D-glucose was observed. The increase in particle-bound β-glucan synthetase activity (UDP-D-glucose:β-1,4-glucan glucosyltransferase), which can be induced by indoleacetic acid in pea-stem tissue, was not prevented by the actions of either actinomycin D or cycloheximide.[78] It was concluded that the rise in activity was induced by activation of a reversibly deactivated enzyme already present. Maize-seedling roots have been incubated *in vivo* with D-[U-^{14}C]glucose and the pattern of incorporation of radioactivity into the polysaccharides of each fraction was investigated.[79] A fucose-containing polysaccharide, characteristic of root slime, was shown to be present only in the membrane system of the root-tip region. Regions of typical, secondary-wall development within the root were characterized by an increased incorporation of radioactivity into D-xylose of polysaccharides within the membrane system. Since in regions of secondary-wall development greater than 60% of all radioactivity was incorporated into a glucan polymer, it was inferred that this polymer, probably cellulose, is not synthesized or transported within the compartments of the membrane system. It was suggested that cellulose synthesis occurs at the surface of the plasmalemma. The biosynthesis of cellulose, hemicelluloses, pectins, and alginates from sugar nucleotides has been reviewed.[80]

Gums, Mucilages, and Pectic Substances

In the light of analytical data acquired for gum exudates from a greatly increased number of *Acacia* species in recent years, the data published for gums from *A. sieberana* and *A. dealbata* are seen to differ considerably in several respects from the range of values established for closely related species.[81] Several specimens of each gum have been re-investigated, and it has been concluded that much of the data published earlier cannot be regarded as typical of these species. A water-soluble gum exudate from *Lannea coromandelica* trees was shown to contain D-galactose (70%), L-arabinose (11%), L-rhamnose (2%), and uronic acids (17%).[82] Three aldobiouronic acids were isolated from acid hydrolysates of the gum and identified as 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-galactose, with the last as the major aldobiouronic acid in the gum. Linkage analysis of the degraded gum gave (chromato-

[78] P. M. Ray, *Plant Physiol.*, 1973, **51**, 609.
[79] D. J. Bowles and D. H. Northcote, *Biochem. J.*, 1972, **130**, 1133.
[80] W. Z. Hassid, in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[81] D. M. W. Anderson, P. C. Bell, G. H. Conant, and C. G. A. McNab, *Carbohydrate Res.*, 1973, **26**, 99.
[82] D. M. W. Anderson and A. Hendrie, *Carbohydrate Res.*, 1973, **26**, 105.

graphic analysis) 3-*O*-β-L-arabinofuranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, 3-*O*-α-D-galactopyranosyl-L-arabinose, 3-*O*-β-D-galactopyranosyl-D-galactose, and 6-*O*-β-D-galactopyranosyl-D-galactose. Structural evidence obtained from methylation analyses and Smith-degradation studies of the whole gum and degraded fractions indicated that the gum molecules are very highly branched, based on a galactan framework consisting of short chains of β-(1 → 3)-linked D-galactose residues, branched and interspersed with β-(1 → 6) linkages. To positions 3 and 6 of this framework are attached either single D-galactose end-groups or short side-chains of D-galactose or of L-arabinose residues, and three aldobiouronic acids. The structure appears to be very similar to that established for *L. humilis* gum. Purified Jeol gum, from *Odina wodier*, contains L-arabinose, D-galactose, and L-rhamnose (35 : 20 : 1), and D-galacturonic acid (14%).[83] Graded hydrolysis of the gum produced 3-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, whilst autohydrolysis of the original gum furnished a degraded polymer containing only D-galactose and D-galacturonic acid residues (3 : 1). On the basis of methylation, periodate oxidation, and Smith-degradation studies, a structure (4) for the repeating unit of the degraded gum was proposed.



(4)

Sapote (*Sapota achras*) gum was shown to contain L-arabinose, in both the furanose and pyranose forms, D-xylose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid.[84] Periodate oxidation of the carboxy-reduced gum gave, *inter alia*, 2-*O*-methyl-D-erythritol and 4-*O*-methyl-D-glucose in amounts suggesting that 37% of the 4-*O*-methyl-D-glucuronic acid residues are unsubstituted in the polysaccharide. Acetolysis of the carboxy-reduced gum gave *O*-α-D-glucopyranosyl-(1 → 2)-(4-*O*-β-D-xylopyranosyl)$_{0,1,2}$-D-xylose, a hitherto undescribed series of oligosaccharides, together with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylose. Methylation studies confirmed that the gum has a highly branched structure. The gum is a member of an uncommon class of plant gums having a D-xylose backbone, and structurally resembles brea gum. Microbial degradation of gum karaya (*Sterculia urens* gum) indicated that the gum contains three different kinds of chains.[85] It was concluded that 50% of the total polysaccharide contains

[83] A. K. Gupta and S. Mukherjee, *Indian J. Chem.*, 1973, **11**, 649.
[84] G. G. S. Dutton and S. Kabir, *Carbohydrate Res.*, 1973, **28**, 187.
[85] W. R. Raymond and C. W. Nagel, *Carbohydrate Res.*, 1973, **30**, 293.

repeating units of four D-galacturonic acid residues containing β-D-galactose branches and having a L-rhamnose residue at the reducing end of the unit. A further 17% of the polysaccharide was demonstrated to contain 50% D-galacturonic acid, 40% L-rhamnose, and 10% D-galactose by weight, and is postulated to contain an oligorhamnan chain, containing D-galacturonic acid branch-residues, and interrupted occasionally by D-galactose units. The D-glucuronic acid residues appear to be confined to the third chain. A g.l.c. method has been proposed as a means of selecting the optimum conditions for the production of glycosylalditols from the Smith-degradation procedure.[86] The method was illustrated using lemon gum. Mucilage polysaccharides containing L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid, and D-xylose have been isolated from the epicarp and mesocarp of coffee cherries.[87] Two polysaccharide fractions were isolated by use of ion-exchange cellulose chromatography. One, a neutral polysaccharide, contained D-galactose and L-arabinose (1 : 4), whereas the other, an acidic heteropolysaccharide, contained D-galactose, L-arabinose, L-rhamnose, and D-galacturonic acid. Partial acidic hydrolysis of the former polysaccharide yielded 3-*O*-D-galactopyranosyl-D-galactose, 3-*O*-D-galactopyranosyl-L-arabinose, and 6-*O*-D-galactopyranosyl-D-galactose. From this evidence, together with periodate-oxidation and methylation studies, it was concluded that the galactoaraban has a highly branched structure, with (1 → 3)-linked L-arabinofuranose residues apparently occurring in the exterior chains, and a core of branched galactan composed of (1 → 6)- and (1 → 3)-linked D-galactopyranose residues. The main component of the mucilage in *Narcissus tazetta* bulbs was shown to be a glucomannan composed of D-glucose and D-mannose residues (2 : 3), and having a relatively low degree of branching.[88] Acetolysis of the polysaccharide furnished β-(1 → 4)- and β-(1 → 3)-linked oligosaccharides composed of D-mannose and/or D-glucose residues. The chain length was determined by methylation analysis to be about 22. Three disaccharides were isolated from partial acidic hydrolysates of plantasan, the seed mucilage of *Plantago major*.[89] Periodate oxidation, methylation, and enzymic studies provided evidence that they are 2-*O*-β-D-xylopyranosyl-D-xylose, 4-*O*-β-D-xylopyranosyl-D-xylose, and 5-*O*-(β-D-glucopyranosyluronic acid)-L-arabinofuranose. The water-soluble carbohydrates of *Zizyphus vulgaris* have been fractionated into an acidic polysaccharide containing L-rhamnose, L-arabinose, D-xylose, D-galactose, and D-galacturonic acid, and a neutral arabinan containing D-galactose and L-arabinose (1 : 30).[90] By the application of classical methods for structural analysis, it was concluded that a chain of (1 → 5)-linked α-L-arabinofuranose residues is contained in a highly branched

[86] G. G. S. Dutton and K. B. Gibney, *Carbohydrate Res.*, 1972, **25**, 99.
[87] J. B. C. Correa and J. D. Fontana, *Anais Acad. Brasil Ciênc.*, 1971, **43**, 803.
[88] K. Kato, Y. Kawaguchi, and T. Mizuno, *Carbohydrate Res.*, 1973, **29**, 469.
[89] M. Tomoda and M. Tanaka, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 989.
[90] M. Tomoda, M. Takahashi, and S. Nakatsuka, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 707.

structure with (1 → 2)-branch points in the arabinan. The water-soluble polysaccharide from the roots of *Polygonum cuspidatum* has a molecular weight of 6000 and contains D-glucose, D-galactose, D-mannose, L-rhamnose, and L-arabinose.[91] The results of partial acidic hydrolysis and methylation studies supported the view that L-arabinose, L-rhamnose, D-glucose, and D-galactose residues occur as non-reducing terminal units, that there are contiguous (1 → 4)-linked β-D-glucose residues, and that some of the D-glucose and D-mannose units are linked through positions 4 and 6. A homogeneous polysaccharide ($[\alpha]_D$ −29°) isolated from *P. falcatum* is composed of D-fructose, D-mannose, D-glucose, and D-galacturonic acid (25 : 10 : 5 : 1).[92] Osmotic-pressure measurements indicated a molecular weight of 4.1×10^5, which was lowered to 1.7×10^5 after liberation of 95% of the D-fructose residues with β-fructofuranosidase. From enzymic degradation and from the production of glycerol on Smith degradation, D-fructose was shown to be present as (2 → 1)- or (2 → 6)-linked ketohexofuranose residues. The presence of (1 → 4)-linked aldohexose units was indicated by the production of erythritol on Smith degradation of the polysaccharide.

Methyl esters of methyl pyranosides of 2,3,4-tri-*O*-methyl-α-D-mannuronic acid, -α-D-glucuronic acid, and -β-D-galacturonic acid underwent rapid β-elimination of the 4-methoxy-group with sodium methoxide in methanol to give 4,5-unsaturated ethers *via cis*, *cis*, and *trans* elimination, respectively.[93] The ease of *cis* elimination to form the 4,5-unsaturated glycosides was explained by ring flexibility, which allows a change of conformation. Only methyl (methyl 2,3,4-tri-*O*-methyl-α-D-mannopyranosid)uronate yielded a 2,3:4,5-di-unsaturated derivative (*trans* elimination) in a much slower reaction, and it was proposed that both eliminations proceeded *via* an *E*1cb mechanism. The results of treatment of pectin with potassium hydroxide solution indicated that the ensuing depolymerization is dependent on the concentration of alkali; there was a facile β-elimination and the ring rigidity of the D-galactopyranosiduronic acid ester dictated a *trans* (*ax*,*ax*) elimination. The action of various bases on three hexuronic acid esters, *viz.* methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate, methyl (methyl 4-*O*-methanesulphonyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate, and methyl (methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate, leading to elimination of the 4-*O*-substituent and to formation of methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)uronate was studied.[94] The effectiveness of the 4-*O*-substituents as leaving groups was methanesulphonyloxy > acetoxy > methoxy. Pectic acid of low molecular weight (10—41 D-galacturonic acid units) has been esterified by aliphatic alcohols.[95]

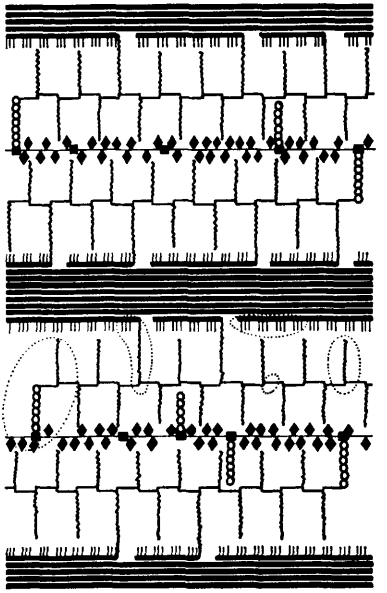
[91] T. Murakami and K. Tanaka, *Chem. and Pharm. Bull.* (*Japan*), 1973, **21**, 1506.
[92] M. Tomoda and S. Nakatsuka, *Chem. and Pharm. Bull.* (*Japan*), 1972, **20**, 2491.
[93] J. N. BeMiller and G. V. Kumari, *Carbohydrate Res.*, 1972, **25**, 419.
[94] G. O. Aspinall and P. E. Barrow, *Canad. J. Chem.*, 1972, **50**, 2203.
[95] M. Manabe, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 185.

The recovered polysaccharides having high degrees of esterification showed decreased molecular weights. A purified, acid-stable *endo*-polygalacturonase was unable to hydrolyse the glycerol ester. The changes occurring in the polysaccharide composition of apple fruits, ripened on and off the tree, have been compared.[96] Neutral carbohydrates associated with pectic extractives decreased, and correspondingly the D-galactose content was lowered, in the detached fruit, whereas both the D-galactose and L-arabinose contents were lowered in fruit on the trees. Soluble polyuronide fractions free from neutral sugars were shown to increase, especially in the detached fruit. Polysaccharides and glycoproteins from apple-fruit cell walls were separated by ion-exchange, cellulose, and gel-permeation chromatographic systems.[97] The presence of linkages existing *in vivo* between these polymers was difficult to establish owing to aggregation after extraction. Differing rates of clarifying activity by purified pectin lyase and *endo*-polygalacturonase on fruit juices from apples and grapes was found to be related mainly to the degree of esterification of the pectin.[98] The pectin in grape juice was considered to exist in various degrees of esterification, depending on the variety and the stage of ripening. Samples from different parts of potato tubers showed that the polyuronide generally has a low degree of esterification (about 40%) and is present in higher concentrations in the cortex and periderm than in the interior tissues.[99] The degree of cross-linking due to calcium and magnesium ions is lowest in the outer layers of the tuber. Partial acidic hydrolysis of an acid polysaccharide from soy sauce resulted in the formation of a degraded polysaccharide, neutral sugars, and D-galacturonic acid and its α-(1 → 4)-linked, homologous di- and tri-saccharides, together with an acid oligosaccharide containing residues of D-galacturonic acid and L-rhamnose.[100] On the basis of structural studies it was suggested that the acid polysaccharide is composed of a backbone of α-(1 → 4)-linked D-galacturonic acid residues and side-chains of D-xylose and *O*-D-galactopyranosyluronic acid-(1 → 2)-L-rhamnose units. In pectin, the interactions with cations was shown to be relatively weak due to the lack of charge on the chains.[101] In contrast to the behaviour of alginates, in which the amplitude diminished with gelation, the broad positive band in the c.d. spectrum of the pectin sol increased in amplitude when chains associated to form a gel. It was proposed that the methyloxycarbonyl constituent of the sol is distributed between a number of rotational states about the C-5—C-6 bond which, owing to different orientations of the carboxy-chromophore in the asymmetric environment of the sugar ring, give rise to bands of varying amplitude. After saponification, the polysaccharide bound Ca^{2+} ions with gel formation and with a

[96] M. Knee, *Phytochemistry*, 1973, **12**, 1543.
[97] M. Knee, *Phytochemistry*, 1973, **12**, 637.
[98] S. Ishii and T. Yokotsuka, *J. Agric. Food Chem.*, 1973, **21**, 269.
[99] D. S. Warren and J. S. Woodman, *J. Sci. Food Agric.*, 1973, **24**, 769.
[100] T. Kikuchi and T. Yokotsuka, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 973.
[101] G. T. Grant, E. R. Morris, D. A. Rees, P. J. C. Smith, and D. Thom, *F.E.B.S. Letters*, 1973, **32**, 195.

large decrease in amplitude of the c.d. band. With the demonstration of ordered chain-associations, it was argued that a two-fold symmetry would be preferred. By use of methylation analysis and purified hydrolytic enzymes, identification and quantitation of the macromolecular components of the cell walls isolated from suspension-cultured sycamore cells (*Acer pseudoplatanus*) have been reported.[102] Pectic polymers, including a neutral arabinan, a neutral galactan, and an acidic rhamnogalacturonan, were isolated by use of an *endo*-polygalacturonase. Structural studies suggested the existence of a branched arabinan and a linear (1 → 4)-linked galactan occurring as side-chains on the rhamnogalacturonan. Small amounts of a xyloglucan, the wall hemicellulose, appeared to be covalently linked to a number of the galactan chains. Thus, the galactan appears to serve as a bridge between the xyloglucan and the rhamnogalacturonan components of the wall. The rhamnogalacturonan was shown to consist of an α-(1 → 4)-linked galacturonan chain that is interspersed with 2-linked rhamnosyl residues. The rhamnosyl residues are not randomly distributed along the chain, but probably occur as *O*-rhamnosyl-(1 → 4)-*O*-galacturonosyl-(1 → 2)-rhamnosyl units. This sequence appears to alternate with a homogalacturonan sequence containing approximately eight residues of 4-linked D-galacturonic acid. About half of the rhamnosyl residues are branched, having a substituent attached to C-4, which is thought to be the likely site of the 4-linked galactan. Xyloglucan or fragments of xyloglucan and acidic fragments from the pectic polysaccharides were released from *endo*-polygalacturonase-pretreated sycamore walls by treatment of these walls with urea, *endo*-glucanase, or sodium hydroxide.[103] The chemical or enzymic treatments required for the release of the xyloglucan from the walls, and its co-chromatography with the acidic pectic substances on ion-exchange columns, indicated that the xyloglucan is covalently linked to the pectic polysaccharide, but is non-covalently bound to the cellulose fibrils of the sycamore cell wall. The structure of this polysaccharide, on the basis of identification of oligosaccharides, is considered to be a repeating heptasaccharide unit consisting of four residues of β-(1 → 4)-linked D-glucose and three residues of terminal D-xylose linked singly and glycosidically to C-6 of three of the D-glucosyl residues. Fragments released by a protease from cell walls pretreated with *endo*-polygalacturonase and *endo*-glucanase provided evidence for a covalent connection between the pectic polysaccharides and the structural protein of the cell wall. Based on these interconnections and the strong binding which occurs between the xyloglucan and cellulose, a tentative structure (5) of the cell wall was proposed.[104] The model is not intended to be quantitative, but wall

[102] K. W. Talmadge, K. Keegstra, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 1973, **51**, 158.
[103] W. D. Bauer, K. W. Talmadge, K. Keegstra, and P. Albersheim, *Plant Physiol.*, 1973, **51**, 174.
[104] K. Keegstra, K. W. Talmadge, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 1973, **51**, 188.



— cellulose elementary fibril

— xyloglucan

— wall protein with arabinosyl tetrasaccharides glycosidically attached to the hydroxyproline residues

— total pectic polysaccharide

— rhamnogalacturonan main chain of the peptic polysaccharide

— arabinan and 4-linked galactan side-chains of the peptic polymer

— 3,6-linked arabinogalactan attached to serine of the wall protein

— unsubstituted seryl residues of the wall protein

(5)

components are in approximately correct proportions. A very similar xyloglucan was isolated and characterized from the walls and in the extracellular medium of suspension-cultures of red kidney-bean (*Phaseolus vulgaris*) cells.[105] Although some minor differences were found, the basic structure of the xyloglucans in the cell walls of these distinctly related species is the same.

Hemicelluloses

Changes in the total hemicellulose composition of leaf and stem tissues of wheat plants have been examined.[106] The content of D-xylose in the total hemicelluloses was found to increase with plant maturity, while those of L-arabinose, D-glucose, and D-glucuronic acid decreased, as did the ratio of β-(1 $\rightarrow$ 3)- to β-(1 $\rightarrow$ 4)-linkages present in the β-glucans. Cell-wall polysaccharides of rye grass (*Lolium perenne*) and *Setaria splendida*, although chemically very similar, were digested to different extents in sheep-feeding experiments.[107] The differences in digestibility between the two grasses could not be explained in terms of the contents of cell-wall polysaccharides, but could result from different digestion of both the hemicellulose and cellulose of each species. When sugar-cane bagasse was partially delignified, it was found that the digestibility of the hemicellulose increased several fold.[72] Addition of sulphate ions to the bagasse digests resulted in an increased consumption of the major polysaccharide constituents of the cell wall (*viz.* arabinan, xylan, and cellulose); this could be due to the ions producing an increased growth rate of one or more microbial species in the rumen or, possibly, to an acceleration of the actions of microbial enzymes on the cell wall.[108] Samples of spear grass (*Heteropogon contortus*), after digestion by bovine rumen, had a higher content of hemicellulose than the fresh grass. The polysaccharides that resisted digestion were fractionated into linear and branched fractions of the hemicellulose B-type.[73] In all cases, the branched hemicellulose was shown to contain more L-arabinose and D-glucuronic acid than the corresponding linear hemicellulose. The rates of hydrolysis of a number of hemicellulose fractions from *H. contortus* have been determined in the presence of extracellular enzymes from bovine-rumen fluid and enzymes liberated by disruption of rumen micro-organisms.[109] From the identity of isolated oligosaccharides, it was confirmed that incomplete digestion of hemicelluloses in the rumen is due to physical protection, possibly by lignin, rather than to structural differences between the hemicellulose components. There was no difference between the rates of digestion of branched and linear hemicelluloses; previous results suggesting such differences can be attributed to the presence of a readily digested glucan in the linear

[105] B. M. Wilder and P. Albersheim, *Plant Physiol.*, 1973, **51**, 889.
[106] A. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1973, **12**, 499.
[107] C. W. Ford, *Austral. J. Biol. Sci.*, 1973, **26**, 1225.
[108] R. F. H. Dekker and G. N. Richards, *Austral. J. Biol. Sci.*, 1972, **25**, 1377.
[109] R. J. Beveridge and G. N. Richards, *Carbohydrate Res.*, 1973, **29**, 79.

hemicellulose fractions. Cell-wall constituents of *Phaseolus aureus* seedlings have shown marked changes during early growth.[76] Considerable changes, both in the quantity of polysaccharides and the sugar composition, in the hemicellulose fraction were noted. Incorporation experiments using D-[^{14}C]glucose showed a higher initial rate of incorporation into hemicelluloses than into α-cellulose. With increasing age, however, the radioactivity was transferred to the cellulose fraction. Hemicelluloses from the flours of *Capsicum annuum* and *C. grossum* have been fractionated into two components, one comprised of D-xylose, L-arabinose, and D-galactose units, and the other containing D-xylose, D-galactose, and D-fructose units.[110] The outer cell-wall glycoproteins of apples are considered to have a possible role in stabilizing hemicellulose structures.[97] Extraction of bamboo shoots with DMSO has yielded a highly branched acid xylan, and an arabinogalactan, and an α-glucan.[111] Changes in the hemicellulose compositions of yam (*Dioscorea rotundata*) tubers [42] and cassava (*Manihot utilissima*) root-tubers [112] during growth have been reported. Polysaccharides from flax seeds, corn cobs, corn roots, and the walls of suspension cultures of *Acer pseudoplatanus* have been shown to contain L-galactose.[113] This was demonstrated by g.l.c. of the TMS derivatives of the sugar components of these polysaccharides after the oxidation of any D-galactose residues with galactose dehydrogenase.

Some important phases in starch and pentosan compositions during the development of wheat and barley kernels have become apparent.[43] The pentosans of wheat were found to develop analogously to starch in one variety and to follow the evolution of crude fibre in another. Crude pentosans from bread have been isolated and fractionated by DEAE-cellulose chromatography into five components.[114] A modified pancreatin method for the purification of water-soluble pentosans from cereals is claimed to preserve the molecular structure of the purified pentosan, compared with previous methods that cause considerable losses of arabinose.[115] Sorghum grains with wide differences in endosperm characteristics also differ significantly in pentosan content.[116] The pericarp is richest in pentosan, followed by the germ and the endosperm. Most pentosans are located in the cell walls of the kernel. The composition and properties of both the water- and alkali-soluble pentosans of sorghum grains were found to be comparable with those of other cereal grains.[117]

An arabinan has been isolated by direct extraction of the bark of trembling aspen (*Populus tremuloides*);[118] the pure polysaccharide has a

[110] A. Soler, C. Perez, and F. Barba, *Anales de Quim.*, 1972, **68**, 1307.
[111] E. Maekawa and K. Kitao, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2445.
[112] A. O. Ketiku and V. A. Oyenuga, *J. Sci. Food Agric.*, 1972, **23**, 1451.
[113] R. M. Roberts and E. Harrer, *Phytochemistry*, 1973, **12**, 2679.
[114] B. L. D'Appolonia, *Cereal Chem.*, 1972, **50**, 27.
[115] C. Lintas and B. L. D'Appolonia, *Cereal Chem.*, 1972, **49**, 731.
[116] A. Karim and L. W. Rooney, *J. Food Sci.*, 1972, **37**, 365.
[117] A. Karim and L. W. Rooney, *J. Food Sci.*, 1972, **37**, 369.
[118] K. S. Jiang and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 499.

DP of 70. Methylation analysis indicated that the arabinan consists of about forty-five (1 → 5)-linked and twenty-five terminal α-L-arabinofuranose residues, which are attached to either C-2 or C-3 or both of these positions in the same sugar residue. The bark arabinan is structurally similar to those isolated from guava fruit, mustard cotyledons, and maritime pine wood. An impure arabinan, consisting of 75% L-arabinose residues, was isolated from the secondary phloem of Scots pine (*Pinus sylvestris*) and, when methylated, gave a polysaccharide consisting of L-arabinose residues only.[119] Its composition is consistent with a main chain of (1 → 5)-linked α-L-arabinofuranose residues, many of which are branched at C-3 and a few at both C-2 and C-3. The endocarp of mango (*Manifera indica*) seeds has been shown to contain a (1 → 4)-linked xylan of approximately seventy-one residues.[39] A xylan extracted from the woody endocarp of the fruit of the Doum palm (*Hyphaene thebaica*) had a DP of 80 and is comprised of a (1 → 4)-linked backbone, with branching at C-3.[120] The ratios of xylose to arabinose in the hemicellulose A and B fractions isolated from corn cobs of differing genetic populations showed variations throughout.[121] The possibility of selecting genetic strains to produce specific cob-hemicellulose compositions was considered. Arabinoxylan preparations from sugar cane showed temperature-induced shifts of optical rotation in aqueous DMSO solution, the sign and magnitude of which depended on the content of arabinofuranose side-groups.[122] From evidence obtained on the shifts on heating and cooling the solutions, together with the sign and magnitude of the shifts, an interpretation was considered in terms of a conformational change of the backbone of β-(1 → 4)-linked D-xylose residues from a random-coil to an ordered, ribbon-like conformation similar to that which exists in the solid state. 3^1-α-L-Arabinofuranosylxylobiose and 3^1-α-L-arabinofuranosylxylotriose have been isolated from rice-straw arabinoxylan after digestion with xylanase.[123] It was shown that the polysaccharide is composed of chains of β-(1 → 4)-linked D-xylopyranosyl residues, with some of these residues bearing α-(1 → 3)-linked L-arabinofuranose side-chains. Treatment of isolated barley aleurone layers with gibberellic acid resulted in a progressive inhibition of cell-wall synthesis.[124] The incorporation of both L-[^{14}C]arabinose and D-[^{14}C]glucose into the cell wall was inhibited by the hormone, synthesis of the pentosan being most affected. Labelling kinetics and pulse-chase analysis indicated that the pentosans are synthesized in the cytoplasm and subsequently transferred to the cell wall. Gibberellic acid inhibited the activity of a membrane-bound arabinosyltransferase present

[119] Y. L. Fu and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 513.
[120] E. S. Amin and A. M. Paleologou, *Carbohydrate Res.*, 1973, **27**, 447.
[121] B. J. Donnelly, J. L. Helm, and H. A. Lee, *Cereal Chem.*, 1973, **50**, 548.
[122] I. C. M. Dea, D. A. Rees, R. J. Beveridge, and G. N. Richards, *Carbohydrate Res.*, 1973, **29**, 363.
[123] S. Takenishi and Y. Tsujisaka, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1385.
[124] K. D. Johnson and M. J. Chrispeels, *Planta*, 1973, **111**, 373.

in the aleurone layers, and this could inhibit synthesis of the cell-wall pentosan.

The holocellulose resulting from acid chlorite-treatment of the secondary phloem of Scots pine (*Pinus sylvestris*) was fractionated and callose was isolated.[125] Structural examination, including partial acidic and enzymic hydrolyses, periodate oxidation, and methylation analysis, indicated that the polysaccharide consists exclusively of β-(1 → 3)-linked D-glucopyranose residues. The stem tissues of mature *Zea mays* contain a β-glucan (DP 127) having (1 → 3)- and (1 → 4)-linked D-glucopyranosyl residues in the molar ratio of 1 : 2.[126] Changes in the levels of activities of various β-glucan hydrolases in the second internode of the stem of the developing oat (*Avena sativa*) plant have been examined, together with concurrent changes in the non-cellulosic β-glucans contained in the corresponding total hemicelluloses.[127] The first of two maximum levels of activity observed for β-glucan hydrolase seemed to be associated with the end of cell-elongation, and only slight changes in the quantity of β-glucan and in the ratio of β-(1 → 3)- to β-(1 → 4)-linkages appear to occur during this period. A second maximum, which is more pronounced for β-(1 → 3)-glucan hydrolase activity, occurred during plant senescence, while hemicellulosic β-glucan was synthesized throughout maturation of the tissue. Possible relationships between the observed changes and the growth and development of the plant tissue were discussed. UDP-D-[^{14}C]glucose at a micromolar level in the presence of magnesium chloride and a particulate enzyme from oat (*A. sativa*) coleoptile, which contains both β-(1 → 3)- and β-(1 → 4)-glucan synthetases, produced a glucan containing mainly β-(1 → 4)-linkages.[128] At high substrate concentrations, activation of the β-(1 → 3)-synthetase was so pronounced that the formation of β-(1 → 3)-D-glucosyl linkages was predominant in the glucan synthesized. In addition to UDP-D-glucose, CDP-D-glucose was found to serve as a substrate for the formation of β-(1 → 3)-glucan in the presence of the β-(1 → 3)-synthetase. Cells from the endosperm of *Lolium multiflorum* grass have been grown in liquid suspension cultures [129] and their walls have been shown to contain large quantities of β-(1 → 3)-(1 → 4)-linked glucan.[130] Particulate fractions from the mid-log phase suspension cultures of *L. multiflorum* endosperm incorporated D-[^{14}C]glucose from UDP-D-[U-^{14}C]glucose into polymeric products, which were fractionated and characterized as β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 3)-(1 → 4)-linked glucans.[131, 132] The proportion of (1 → 3)- and (1 → 4)-linkages in the total products varied with the concentration of UDP-D-glucose. At 1 μmol l^{-1} UDP-D-glucose, 90% of the

[125] Y. L. Fu, P. J. Gutmann, and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 507.
[126] A. J. Buchala and H. Meier, *Carbohydrate Res.*, 1973, **26**, 421.
[127] A. J. Buchala and H. Meier, *Planta*, 1973, **111**, 245.
[128] C. M. Tsai and W. Z. Hassid, *Plant Physiol.*, 1973, **51**, 998.
[129] M. M. Smith and B. A. Stone, *Austral. J. Biol. Sci.*, 1973, **26**, 123.
[130] M. M. Smith and B. A. Stone, *Phytochemistry*, 1973, **12**, 1361.
[131] M. M. Smith and B. A. Stone, *Biochim. Biophys. Acta*, 1973, **313**, 72.
[132] M. M. Smith and B. A. Stone, in 'Biochemistry of the Glucosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972, p. 395.

residues had (1 → 4)-linkages, but this proportion fell to 49% at 1 mmol l^{-1} concentrations. A water-soluble polysaccharide, which was the predominant product at 100 mmol l^{-1} UDP-D-glucose, was shown to be a β-(1 → 3)-(1 → 4)-glucan similar to the glucan present in the cell walls of *Lolium* endosperm. The activities of two microsomal β-glucan synthetase systems were found to be altered as a result of the formation of a microsome-bound fraction of low molecular weight, which increased with time as the injured etiolated cotton hypocotyls were exposed to air.[77] This resulted in a five-fold increase in the ratio of non-cellulosic to cellulosic D-glucosyl linkages synthesized from UDP-D-glucose.

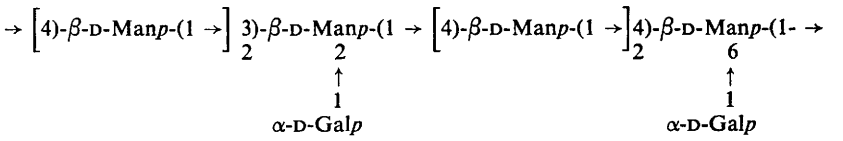
Four fructans isolated from the rhizomes of *Polygonatum odoratum* were shown to possess a non-reducing, linear structure of (2 → 1)-linked β-D-fructofuranose residues, with one D-glucose residue linked in the middle of the molecule as in *neo*-kestose.[133] With a particulate enzyme preparation from *Phaseolus aureus* hypocotyls, UDP-D-[U-^{14}C]galactose served as a precursor for a number of the products, one of which was characterized as an alkali-soluble, β-(1 → 4)-linked galactan.[134] The labelling patterns of the [^{14}C]oligosaccharides derived from acetolysis of the galactan indicated that only slightly more than two D-[^{14}C]galactose moieties were added, on average, to the growing polysaccharide chain, and that the additions took place at the reducing-end of the chain. An alkali-soluble polysaccharide containing D-galactose was also isolated, but it appeared to have a different structure, as judged by the radioactive oligosaccharides liberated on acetolysis. The reserve polysaccharide of palm (*Erythea edulis*) kernels has been characterized as a β-(1 → 4)-mannan.[135] A commercially available isoprenol appears to substitute for the endogenous mannosyl acceptor in *P. aureus* enzyme preparations.[136] An assay that can be used in the isolation and purification of GDP-D-mannose:lipid phosphate transmannosylase was also reported, together with data supporting earlier suggestions as to the probable nature of the lipid phosphate acceptor. A particulate enzyme fraction from cotton fibres catalysed the incorporation of D-mannose from GDP-D-[U-^{14}C]mannose and of D-glucose from UDP-D-[U-^{14}C]glucose into acid lipids.[137] The kinetics of incorporation suggested a turnover of lipid and the properties of the mannolipid were shown to be similar to a previously isolated mannosylphosphorylundecaprenol.[137a] The active lipid fraction from cotton fibres has been isolated and purified.[138] The active components had chromatographic properties similar to those reported for ficaprenyl phosphate;

[133] M. Tomoda, N. Satoh, and A. Sugiyama, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1806.
[134] N. Panayotatos and C. L. Villemez, *Biochem. J.*, 1973, **133**, 263.
[135] D. Robic and F. Percheron, *Phytochemistry*, 1973, **12**, 1369.
[136] A. F. Clark and C. L. Villemez, *F.E.B.S. Letters*, 1973, **32**, 84.
[137] W. T. Forsee and A. D. Elbein, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 930.
[137a] M. Scher, W. J. Lennarez, and C. C. Sweeley, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **59**, 1313.
[138] W. T. Forsee and A. D. Elbein, *J. Biol. Chem.*, 1973, **248**, 2858.

indeed, synthetic ficaprenyl phosphate was effective in replacing the requirement for the active cotton lipid-acceptor. Glucose, mannose, and significant proportions of arabinose and galactose were released on acidic hydrolysis of the purified acidic lipid isolated from intact cotton bolls.

An equation based on the principle of optical superposition, previously derived for native xylans, has been adapted and applied to seed galactomannans.[139] The specific rotations for aqueous solutions of the galactomannan obeyed the relationship with the ratio between monosaccharide components of the polysaccharides required by the equation. Similar relationships were demonstrated for the optical rotations of solutions of galactomannans in alkali and of methylated galactomannans in chloroform. Within naturally related groups of galactomannan-containing plant species, the mannose-to-galactose ratios of the polysaccharide varied between relatively narrow limits. The ratio of galactose to mannose in the reserve galactomannans of the seeds of the Leguminosae was shown to reflect their classification within the family.[140] Examination of polysaccharides from the seeds of two species in each of the genera *Medicago*, *Melilotus*, *Trifolium*, and *Trigonella* from the tribe Trifolieae showed that, with the exception of that from *Trigonella cretica*, all the polysaccharides have the same proportion of galactose to mannose. Six other *Trigonella* sp. were investigated and their galactomannans were found to correspond with those from the other species of Trifolieae studied, but not with that from *T. cretica*. It was suggested that this and similar studies may aid in the classification of Leguminosae. In the earliest stages of germination of the seeds of *Trigonella foenum-graecum*, no change in the main reserve carbohydrate (a galactomannan) was observed.[141] This endosperm galactomannan was mobilized only after the emergence of the radicle, and within one day was completely degraded to mainly D-galactose and D-mannose. Mobilization of the galactomannan is accompanied by formation in the endosperm of a dissolution zone, the form of which implied that the aleurone layer is involved in the degradation process. From studies of the breakdown of galactomannans of fenugreek (*T. feonum-graecum*), crimson clover (*T. incarnatum*), and lucerne (*Medicago sativa*) in endosperms devoid of the embryo, it was concluded that the cells of the aleurone layer are responsible for the synthesis and the secretion into the storage cells of the enzymes necessary for degradation of the galactomannan.[142] The physiology of galactomannan breakdown was compared with and contrasted to that of starch mobilization in the endosperm of germinating cereal grains. A water-soluble galactomannan isolated from the seeds of *Cassia occidentalis* was shown to contain residues of D-galactose and D-mannose (1 : 3).[143] A tentative structure (6) has been

[139] P. Kooiman, *Carbohydrate Res.*, 1972, **25**, 1.
[140] J. S. G. Reid and H. Meier, *Z. Planzenphysiol.*, 1970, **62**, 89.
[141] J. S. G. Reid, *Planta*, 1971, **100**, 131.
[142] J. S. G. Reid and H. Meier, *Planta*, 1972, **106**, 44.
[143] D. S. Gupta and S. Mukherjee, *Indian J. Chem.*, 1973, **11**, 505.



(6)

assigned to a galactomannan from *C. absus* seeds on the basis of information obtained by methylation and periodate oxidation.[144] Two glucomannans were isolated from the tubers of *Arum orientale* after fractionation by gel-permeation chromatography.[145] One of the polysaccharides is composed of D-glucose and D-mannose residues (2 : 3), and evidence obtained from i.r. spectroscopy and by periodate oxidation indicated the presence of (1 → 4)-β-D-glucopyranosyl linkages. An ultracentrifugally homogeneous glucomannan acetate derived from konjac mannan has been subjected to acetolysis.[146] In addition to the isolation of β-(1 → 4)-linked oligosaccharides composed of D-mannose and/or D-glucose, the three oligosaccharides (7)—(9), corresponding to branch points in the polysaccharide, were identified.

β-D-Man*p*-(1 → 3)-D-Man*p*

(7)

β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 → 3)-D-Man*p*

(8)

β-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-Glc*p*

(9)

The glucomannan of the tubers of orchids, *Orchis morio*, *O. mascula*, and *Platanthera bifolia*, is mobilized in early stages of growth by degradation to mannotriose, mannobiose, and D-mannose.[147] D-Mannose is rapidly converted into sucrose, which is then transported to the young tubers and used for the synthesis of new glucomannan. The old tubers were found to contain UDP-D-glucose and UDP-D-galactose, whereas UDP-D-glucose, UDP-D-galactose, GDP-D-mannose, and ADP-D-glucose were found in new tubers. A glucomannan has been isolated from the chlorite holocellulose from *Pinus radiata*.[148] From the results of methylation analysis, it was proposed that the polysaccharide (molecular weight 9.3×10^3) is composed of a linear chain of forty-five (1 → 4)-linked β-D-glucopyranose and β-D-mannopyranose units, with D-mannose as the non-reducing end-group. D-Galactopyranose units are attached to the chain, probably through an α-(1 → 6)-linkage, in the ratio of one D-galactose residue to

[144] V. P. Kapoor, *Indian J. Chem.*, 1973, **11**, 13.
[145] C. Z. Achtardjiev and M. J. Koleva, *Phytochemistry*, 1973, **12**, 2897.
[146] K. Kato and K. Matsuda, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2045.
[147] G. Franz and H. Meier, *Plant Medica*, 1971, **19**, 326.
[148] V. D. Harwood, *Svensk Papperstidn.*, 1973, **76**, 377.

every seventy-five sugar residues in the chain. The distribution and amounts of *O*-acetyl groups on the 2- and 3-positions of the D-mannose residues of a sample of native *P. sylvestris* (Scots pine) were found to be in agreement with those previously reported.[149] The polysaccharide also contains approximately 5% of terminal D-galactose residues, linked to the 6-positions of the D-mannose and D-glucose residues, showing that it is actually a galactoglucomannan, as are probably all softwood polysaccharides generally referred to as glucomannans.

A galactoglucomannan isolated from *P. sylvestris* by sodium hydroxide-borate extraction of the secondary phloem has been shown to consist of a linear chain of at least sixty β-(1 → 4)-linked D-glucopyranose and D-mannopyranose residues, with approximately 3% of the hexose units substituted at C-6 with an α-D-galactopyranose residue.[150] An electrophoretically homogeneous, water-soluble polysaccharide isolated from the bark of trembling aspen (*Populus tremuloides*) has been shown to contain D-galactose, D-glucose, and D-mannose residues (1 : 2 : 2.6).[151] Polysaccharides isolated from the seeds of seven species of Liliacaea and two species of Iridaceae appeared to be galactoglucomannans.[152] One of the polysaccharides, from the endosperm of *Asparagus officinalis*, was shown to consist of a linear chain of β-(1 → 4)-linked D-mannose and D-glucose residues to which are attached single D-galactose side-chains linked (1 → 6), and probably with the α-configuration. Methylation analysis suggested that this polysaccharide consists of α-D-galactopyranose units linked to C-6 of, on average, every fifth or sixth D-glucose or D-mannose residue of the main chain. A polysaccharide containing D-galactose, D-mannose, D-glucose, and D-xylose (2 : 7 : 2 : 1), which was isolated from the seeds of *Cassia tora*, appears to be highly branched and probably contains α-linked D-galactopyranose and D-xylopyranose end-groups attached to (1 → 4)-linked β-D-mannopyranose and β-D-glucopyranose units.[153] The 'wood' of the tropical liliaceous tree, *Cordyline indivisa*, contains a glucomannan possessing a main chain of β-(1 → 4)-linked D-mannose and D-glucose residues to some of which D-galactose and D-xylose residues are probably attached at position 6.[154] Extraction of the cell walls of *Lolium multiflorum* grass with DMSO yielded a complex mixture of polysaccharides, containing D-glucose, D-xylose, L-arabinose, and D-galactose, which are probably covalently linked to lignin.[155] Fractionation of the complexes yielded a major component (molecular weight 1.5×10^5), which was apparently homogeneous by gel-permeation chromatography. The carbazole reaction has been reported to be suitable for the measurement

[149] B. Lindberg, K. G. Rosell, and S. Svensson, *Svensk Papperstidn.*, 1973, **76**, 383.
[150] Y. L. Fu and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 517.
[151] K. S. Jiang and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 503.
[152] N. Jakimow-Barras, *Phytochemistry*, 1973, **12**, 1331.
[153] S. C. Varshney, S. A. I. Rizvi, and P. C. Gupta, *J. Agric. Food Chem.*, 1973, **21**, 222.
[154] R. Sieber, *Phytochemistry*, 1972, **11**, 1433.
[155] I. M. Morrison, *Phytochemistry*, 1973, **12**, 2979.

of uronic acids in lignin–carbohydrate complexes.[156] Examination of such a complex by ^1H n.m.r. spectroscopy has prompted the suggestion that the carbohydrate is attached to the hydroxypropyl side-chain of lignin, possibly at the α-position to the guaiacyl or syringyl units. The nature of the complex was studied using partial acidic hydrolysis, enzymic hydrolysis, and periodate oxidation; the existence of a highly branched polysaccharide, possibly of the xylan or arabinan type, attached to the lignin either at a non-benzylic position or possibly at a sterically hindered benzylic position was suggested.[157]

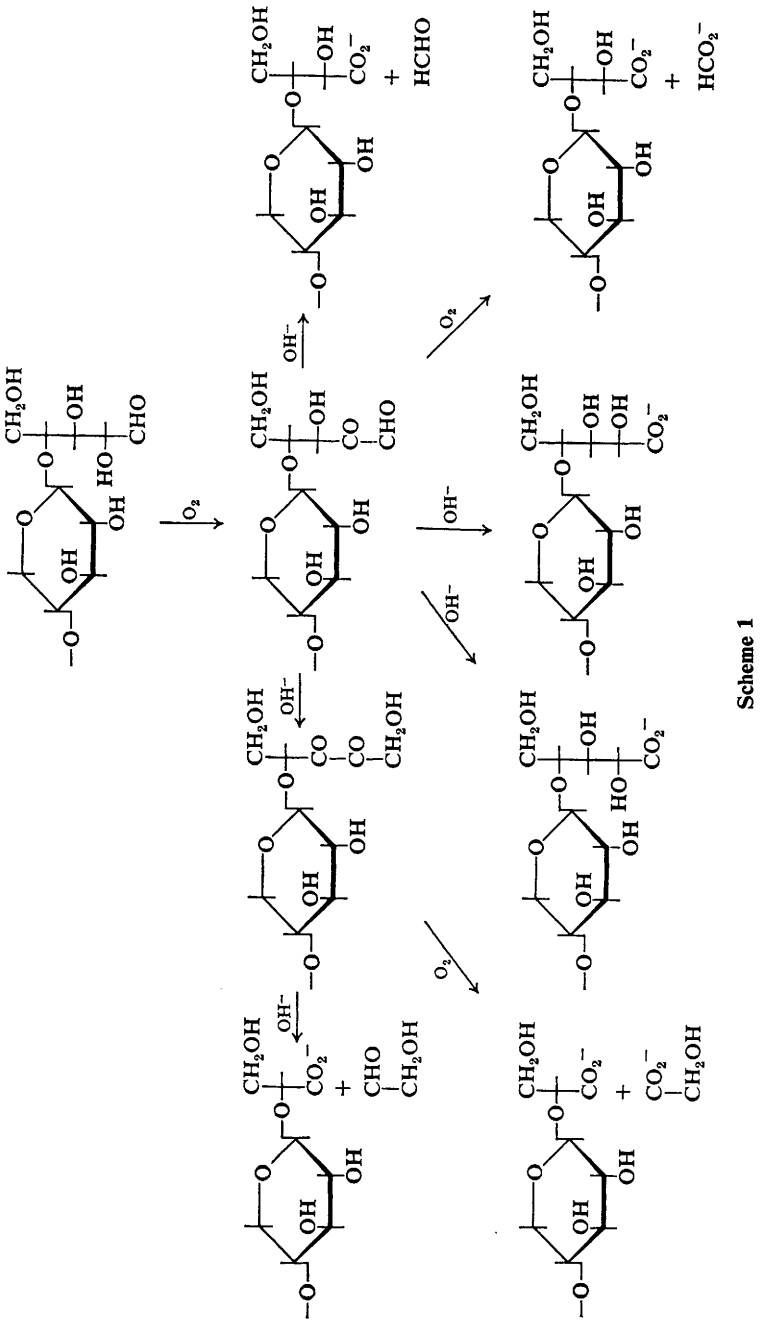
A method for estimation of the uronic acid content of polysaccharides depends on reduction of the polymer with sodium borohydride, esterification of carboxy-groups with propene oxide and diazomethane, followed by reduction with sodium borotritide.[158] Tritiated D-glucose and 4-*O*-methyl-D-glucose were then counted in hydrolysed samples using scintillation methods. Methods, utilizing a minimum number of analyses, have been suggested for obtaining approximate compositional data for woods, especially for closely related species.[159] The optimum separation of the constituent sugars liberated on acidic hydrolysis of the whole wood was obtained by g.l.c. but, in order to translate the analytical data into results that were meaningful with respect to the wood polysaccharides, it was necessary to isolate the 4-*O*-methylglucuronoxylan and glucomannan components, by extraction of the holocellulose with aqueous alkali, to obtain the ratios of constituent carbohydrate units. As part of a study concerned with the isolation and characterization of hemicelluloses from paper pulps, hemicelluloses were isolated from pulps of bleached hardwood and rice straw.[160] Both sources had high contents of pentosan, but the wood hemicellulose contained a higher proportion of material containing uronic acid. It has been shown that raising of the hemicellulose content by addition is more effective in promoting the strength of paper than raising the hemicellulose *in situ* in the pulp.[161] The use of hemicelluloses as an additive increased the hemicellulose concentration on the external-fibre surfaces, where it could exert its maximum effect, and the extents of retention of hemicelluloses and improvement of strength depended on the types of hemicellulose fraction and pulp used. The topochemical pattern of delignification of black spruce (*Picea mariana*) appeared to be closely associated with the removal of hemicelluloses during the initial stages of delignification.[162] Removal of hemicellulose allowed a

[156] J. W. T. Merewether, L. A. M. Samsuzzaman, and I. C. Calder, *Holzforschung*, 1972, **26**, 180.
[157] J. W. T. Merewether, L. A. M. Samsuzzaman, and R. G. Cooke, *Holzforschung*, 1972, **26**, 193.
[158] A. J. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1973, **12**, 655.
[159] C. M. Stewart, J. F. Melvin, S. H. Tham, and E. Zerdoner, *Cellulose Chem. Technol.*, 1973, **7**, 371.
[160] A. E. El-Ashmawy, F. Mobarak, and Y. Fahmy, *Cellulose Chem. Technol.*, 1973, **7**, 315.
[161] F. Mobarak, A. E. El-Ashmawy, and Y. Fahmy, *Cellulose Chem. Technol.*, 1973, **7**, 325.
[162] J. R. Wood, P. A. Ahlgren, and D. A. I. Goring, *Svensk Papperstidn.*, 1972, **75**, 15.

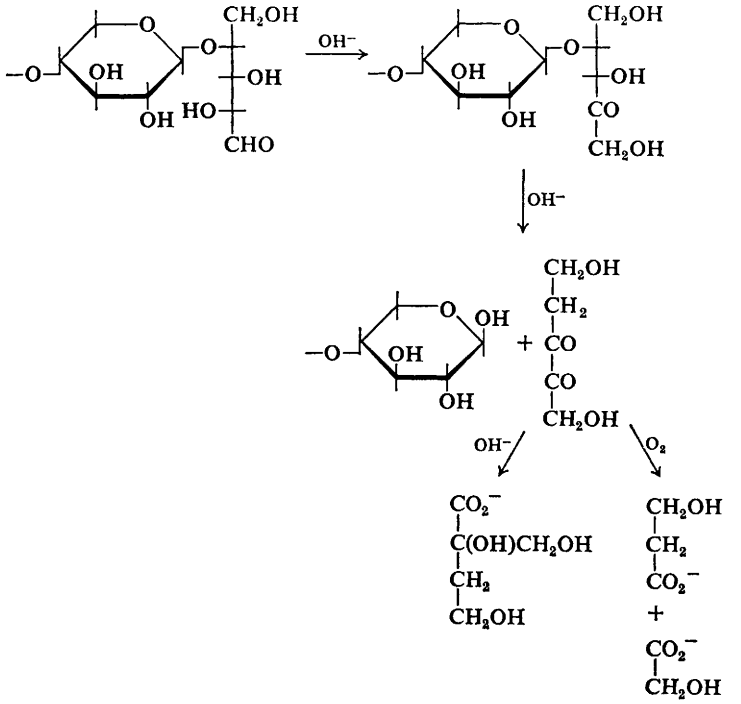
preferential dissolution of lignin from the secondary wall, and the effect was considered to be related either to differences between the chemical reactivity of lignin in the compound middle lamella and secondary wall of the cell or to differences in the porous structure of these morphological regions. The adsorption of D-xylose from aqueous solutions by the holocellulose of *Terminalia superba* wood has been studied, and the type of adsorption processes taking place and the influence of the adsorbed D-xylose upon the swelling and dissolution of the holocellulose in alkali were determined.[163]

A water-soluble polysaccharide from grapes has been reported to contain both D-glucuronic and D-galacturonic acids.[164] Structural studies on a 4-*O*-methylglucuronoxylan from *Cordyline indivisa* have shown that the polysaccharide has a linear backbone of β-(1 → 4)-linked D-xylose residues and, on average, each molecule (DP 142) carries eight or nine 4-*O*-methyl-D-glucopyranosyluronic acid residues linked α-(1 → 2) to D-xylose.[154] Hemicellulose extracted from the chlorite holocellulose of sabai grass (*Eulaliopsis binata*) was shown to contain 87% D-xylose and 13% D-glucuronic acid.[165] Hydrolysis of the fully methylated hemicellulose yielded 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-glucuronic acid (4.5 : 40 : 1 : 5) which, coupled with an estimated DP of 177, indicated that the polysaccharide consists of a continuous chain of (1 → 4)-linked D-xylopyranose residues with single unit branches of D-glucopyranosyluronic acid attached to one out of every nine D-xylose units by a (1 → 2)-linkage. The xylan from birch wood, *Betula verrucosa*, was degraded along the xylan chains during treatment with oxygenated alkali under conditions simulating oxygen-bleaching of wood pulp.[166] Determinations of the end-groups showed that lyxonic, xylonic, threonic, and glyceric acids were the major, terminal, acid constituents, and that large amounts of reducing end-groups (xylose and isomerization products thereof) were present. Part of the uronic acid substituents were split off during this treatment and a small amount of 4-*O*-methyl-D-glucuronic acid was detected in the solution, together with larger quantities of formic, glycolic, 3-hydroxypropionic, 3-deoxy-2-*C*-hydroxymethyltetronic, and 2,4-dihydroxybutyric acids. Based upon the analysis of the recovered xylan and the products split off, a scheme demonstrating the main reaction paths was presented (see Scheme 1). The results showed that the reactions are very similar to those occurring during the degradation of cellulose by oxygenated alkali. Two previously unknown diastereoisomeric 3,6-dideoxyhexonic acids were present in large amounts after the alkali treatment, both in the presence and in the absence of oxygen, and the mechanism proposed for their formation is shown in Scheme 2.

[163] M. Nedelcheva, S. Gabir, and S. Bencheva, *Cellulose Chem. Technol.*, 1972, **7**, 171.
[164] V. I. Zinchenko, G. M. Keshisheva, and I. G. Yazlovetsky, *Priklad. Biokhim. Mikrobiol.*, 1973, **9**, 99.
[165] S. R. D. Guha, J. Nand, and J. S. Negi, *Indian J. Chem.*, 1973, **11**, 653.
[166] H. Kolmodin and O. Samuelson, *Svensk Papperstidn.*, 1973, **76**, 71.



Scheme 1



Scheme 2

An alkali-soluble xylan containing both 4-*O*-methyl-D-glucopyranosyluronic and D-galactopyranosyluronic acids has been isolated from *B. verrucosa*.[167] It was concluded that the D-galacturonic acid moieties are integral parts of the xylan structure and that they are linked to non-reducing, terminal D-xylose units in the highly branched polysaccharide. The distribution of *O*-acetyl groups in the naturally acetylated xylan of *B. verrucosa* has been determined.[168] The proportions of non-acetylated D-xylose residues and those acetylated in the 2-, 3-, and 2,3-positions, respectively, were found to be 44 : 24 : 22 : 10; this does not correspond to the equilibrium distribution established by *O*-acetyl migration. Some migration of *O*-acetyl groups almost certainly occurred and it was suggested that acetylation of the 2-position predominates under biological conditions. Appreciable amounts of hemicelluloses, including arabinogalactans, galactoglucomannans, and 4-*O*-methylglucuronoxylan, are present in neutral, sulphite-pulped Scandinavian spruce (*Picea abies*).[169] Hydrolysis of the hemicellulose gave rise to a previously unreported dimer

[167] K. Shimizu and O. Samuelson, *Svensk Papperstidn.*, 1973, **76**, 150.
[168] B. Lindberg, K. G. Rosell, and S. Svensson, *Svensk Papperstidn.*, 1973, **76**, 30.
[169] K. Shimizu and O. Samuelson, *Svensk Papperstidn.*, 1973, **76**, 156.

of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, showing that a substantial proportion of 4-*O*-methyl-D-glucuronic acid moieties is located on adjacent D-xylose units in the isolated xylan. The presence of D-xylonic, D-mannonic, and D-galactonic acids in the hydrolysates indicated that end-group oxidation of the hemicellulose occurred even on extraction of the pulp under neutral conditions.

Bark of the quaking aspen (*Populus tremuloides*) has been shown to contain a 4-*O*-methylglucuronoxylan which is almost identical in structure to that isolated from the wood of the tree; the bark polysaccharide contains a linear, β-(1 → 4)-linked, D-xylose backbone with 4-*O*-methyl-α-D-glucuronic acid residues attached to C-2 of the backbone.[170] A 4-*O*-methylglucuronoxylan has been isolated from the bark of the white willow (*Salix alba*) and was reported to have a linear backbone of 171 β-(1 → 4)-linked D-xylopyranose residues with, on average, every ninth D-xylose unit carrying a single 4-*O*-methyl-α-D-glucopyranosyluronic acid residue linked to C-2.[171]

The fundamental segment of naturally occurring 4-*O*-methylglucuronoxylans, the aldotriouronic acid trihydrate *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1 → 2)-*O*-β-D-xylopyranose-(1 → 4)-D-xylopyranose, has been studied by *X*-ray crystallography.[172] The orientation of the trisaccharide molecule and hydrogen-bonding pattern are such that there appears to be a planar aldobiouronic-acid 'polymer' along the *c*-axis and a helical xylan 'polymer' along the *b*-axis, with three water molecules of crystallization clustered between the perpendicular chains and filling in an otherwise open structure. Thermal degradation studies on xylans and related model compounds showed that the pyrolysis of *O*-acetyl-4-*O*-methylglucuronoxylan, 4-*O*-methylglucuronoxylan, and β-D-xylopyranosides involves cleavage of the glycosidic bonds, with the resulting glycosyl units partly forming random condensation products and partly being degraded to a variety of volatile products.[173] A complex polysaccharide composed of L-arabinose, D-xylose, and D-glucuronic acid (9 : 8 : 1) has been isolated from mature stalks of the reed *Arundo donax*.[174] From the results of methylation analysis, which showed the presence of 2,3,4-tri-, 2,3-di-, 2- and 3-mono-*O*-methyl-D-xyloses, and 2,3,5-tri-*O*-methyl-L-arabinose, it was concluded that this essentially linear polysaccharide has structural features similar to those of polysaccharides isolated from other *Gramineae* and from bamboo culms,[175] and from the stalks of *Cyperus papyrus*,[176] and the leaves and stalks of *Zea mays*.[177] An arabinogalacto(4-*O*-methylglucurono)xylan isolated from barley leaves (*Hordeum vulgare*) was reported

[170] K. S. Jiang and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 493.
[171] R. Toman, *Cellulose Chem. Technol.*, 1973, **7**, 351.
[172] R. A. Moran and G. F. Richards, *Carbohydrate Res.*, 1972, **25**, 270.
[173] F. Shafizadeh, G. D. McGinnis, and C. W. Philpot, *Carbohydrate Res.*, 1972, **25**, 23.
[174] F. Barnoud, G. G. S. Dutton, and J. P. Joseleau, *Carbohydrate Res.*, 1973, **27**, 215.
[175] E. Maekawa and K. Kitao, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2073.
[176] A. J. Buchala and H. Meier, *Phytochemistry*, 1972, **11**, 3275.
[177] G. G. S. Dutton and M. S. Kabir, *Phytochemistry*, 1972, **11**, 779.

to have a main chain of β-(1 → 4)-linked D-xylopyranose units to which are attached residues of L-arabinofuranose, (1 → 4)-linked D-galactopyranose, D-xylopyranose-(1 → 2)-L-arabinofuranose, and 4-*O*-methyl-D-glucopyranosyluronic acid.[178]

Algal Polysaccharides

Agar.—The effects of the concentration of agarose, DMSO, and substituted agaroses on the mechanism of gel formation have been evaluated with reference to the 'network theory of gel formation'.[179] Factors affecting the formation of the agarose–iodine colour complex have led to the suggestion that the iodine molecules are incorporated between the agarose helices in the gel-II state. A new analytical method has been developed that is capable of determining the molar contents of 3,6-anhydrogalactose and galactose residues, and of 6-*O*-methyl, 6-sulphate, and pyruvic acid groups in agar-type polysaccharides by analysis of the 100 MHz ^{1}H n.m.r. spectra of partial acidic hydrolysates.[180, 181] The analytical results obtained for the polysaccharides from *Gelidium amansii*, *Gracilaria verrucosa*, and *Gloiopeltis furcata* were in agreement with those obtained by other methods.

Alginic Acid.—Radioautographic and radioisotopic analyses have provided evidence that alginic acid is polymerized in the cytoplasm by unpigmented particles, which take up the basic dye Toluidine Blue O.[182] A systematic approach to stereochemical aspects of the conformational analysis of polyguluronic acid, polymannuronic acid, cellulose, and mannan, by comparison of the flexibility of these polysaccharides, has indicated that the polyuronic acids are more flexible than the corresponding glycans.[56] C.d. evidence has been obtained for the involvement of carboxylate *n*-orbitals of contiguous α-L-guluronate residues in the specific binding of calcium ions, which occurs co-operatively with the sol-to-gel transition for alginate.[183] Interactions between alginates and bivalent cations of calcium and strontium have been studied by this c.d. spectroscopic technique and by computer model-building, which have led to proposals summarized in terms of an 'egg-box model'.[101] Light-scattering and viscosity experiments showed that the relative extensions for the three types of 'blocks' in alginate increases in the order 'MG-blocks' < 'MM-blocks' < 'GG-blocks'.[184] From a comparison with the calculated free-rotational dimensions, it was concluded that this effect is due to differences in hindrance to rotation about the glycosidic linkages in the different blocks. Calculations by statistical mechanics yielded the unperturbed dimensions, which are in

[178] A. J. Buchala, *Phytochemistry*, 1973, **12**, 1373.
[179] N. M. K. Ng Ying Kin and W. Yaphe, *Carbohydrate Res.*, 1972, **25**, 379.
[180] K. Izumi, *Carbohydrate Res.*, 1973, **27**, 278.
[181] K. Izumi, *Biochim. Biophys. Acta*, 1973, **320**, 311.
[182] G. G. Leppard, *Canad. J. Botany*, 1973, **51**, 957.
[183] E. R. Morris, D. A. Rees, and D. Thom, *J.C.S. Chem. Comm.*, 1973, 245.
[184] O. Smidsrød, R. M. Glover, and S. G. Whittington, *Carbohydrate Res.*, 1973, **27**, 107.

agreement with the above order only when the L-guluronic acid residues are assumed to adopt the $1C$ conformation. The *X*-ray fibre-diffraction pattern of alginic acid (obtained on bundles of fibres prepared from *Fucus vesiculosus*) has been indexed to an orthorhombic unit cell having $a = 0.76$, b(fibre axis) $= 1.04$, and $c = 0.86$ nm.[185] A sheet-like structure, involving one intra-chain, one intra-sheet, and one inter-sheet hydrogen bond per monosaccharide residue, was proposed. An empirical measure of the molecular stiffness of periodate-oxidized alginates, both before and after reduction with sodium borohydride, showed that, whereas the stiffness decreased very sharply with increasing degrees of oxidation, it changed very little on subsequent reduction of the products.[186] From this and the known structures of the inter-residue hemiacetals in the oxidized products, it was inferred that the increase in flexibility brought about by oxidation is specifically associated with the rotation about the three bonds adjoining C-4 of the oxidized hexuronic acid residues. This rotation is considerably less hindered than that around the glycosidic linkages, both in the intact alginate and in the partially oxidized and reduced products. The uronic acid sequences of several alginate samples have been determined by cleaving the alginates into alternating and homopolymeric forms by partial acidic hydrolysis, after which the relative proportion of blocks of D-mannuronic and L-guluronic acids in the homopolymeric fraction was determined by ^{1}H n.m.r. spectroscopy.[187] The alginates examined in this way fall into two main classes: those containing a high percentage of L-guluronic acid blocks with intermediate amounts of alternating regions and small amounts of D-mannuronic acid blocks, and those containing a small proportion of L-guluronic acid blocks and approximately equal proportions of D-mannuronic acid and alternating blocks. It was suggested that, in the homopolymeric regions of the alginate chain, D-mannuronic acid residues are β-linked in the $1C$ conformation and L-guluronic acid residues are α-linked in the $1C$ conformation.

Carrageenan.—Total carrageenan levels of four *Gigartina* species showed little variation between male, female, and tetrasporic plants.[188] However, whereas male and female gametophyte plants gave carrageenans with $\kappa : \lambda$ ratios usually ranging from 1.0 to 4.0, tetrasporophyte carrageenans gave very low ratios, indicative of a virtual absence of κ-carrageenan from plants at this stage of the life cycle. Examination of the morphologically similar sporophytic and gametophytic plants of *Chondrus crispus* showed that the former contains λ-carrageenan, and that the gametophytes contain χ- and two additional carrageenans, which are soluble in solutions of potassium chloride.[189] After alkaline modification, the salt-soluble

[185] E. D. T. Atkins, I. A. Nieduszynski, W. Mackie, K. D. Parker, and E. E. Smolko, *Biopolymers*, 1973, **12**, 1865.
[186] O. Smidsrød and T. Painter, *Carbohydrate Res.*, 1973, **26**, 125.
[187] A. Penman and G. R. Sanderson, *Carbohydrate Res.*, 1972, **25**, 273.
[188] S. E. Pickmere, M. J. Parsons, and R. W. Bailey, *Phytochemistry*, 1973, **12**, 2441.
[189] E. L. McCandless, J. S. Craigie, and J. A. Walter, *Planta*, 1973, **112**, 201.

components were separated into a soluble carrageenan and a gel, which was indistinguishable from χ-carrageenan and was presumably derived from μ-carrageenan. The salt-soluble polysaccharide, which possesses a unique i.r. spectrum differing from that of alkali-modified λ-carrageenan, appears to represent a third type of carrageenan in the gametophytes. Different patterns of sulphation of the galactans were observed in the separate plants; the sulphate groups are present at C-2 of the precursor in the sporophytes, whereas the gametophytes appear to add them principally at the C-4 position. An ι-carrageenan has been selectively cleaved at the D-galactose 6-sulphate 'kink points' to give short-chain segments that did not gel, but which gave an optical rotation–temperature curve characteristic of gelatin.[190] The molecular weight of these segments changed with temperature, and the shift in optical rotation was paralleled by a dimerization, thus confirming the suggestion of a coil-to-double-helix transition. Light-scattering measurements showed a behaviour typical of concentration-dependent aggregation in both states. Conformational aspects of λ- and κ-carrageenans in solution, as indicated by induced Cotton effects in the Methylene Blue complexes, showed that the dye bound to the κ-form to give an excitation-like band that correlated with the proposed right-handed double helix.[191] The induced c.d. band for the Methylene Blue complex of the λ-form was negative, thus demonstrating a qualitative difference in the preferred conformation of this polymer. Comparison of these results with those of similar studies on the glycosaminoglycans revealed general patterns of conformational preferences among these polymers.

ι-Carrageenan has been characterized by chemical methods to provide a firm basis for the interpretation of *X*-ray diffraction and optical data, and so to confirm the carrageenan double helix.[192] The polysaccharide was isolated from *Eucheuma spinosum* and was shown by methylation analysis, partial fragmentation, and other evidence to have a masked repeating structure in which D-galactose 4-sulphate and 3,6-anhydro-D-galactose 2-sulphate residues are arranged alternately in linear chains, with formal replacement of approximately one in every ten anhydro-sugar residues by D-galactose 2,6-disulphate. Treatment with alkaline borohydride converted this structure into a genuinely alternating copolymer. A polysaccharide from *E. cottonii* has similar alternating 1,4- and 1,3-linked residues, but is distinctive in that it has no detectable 2-sulphate and, therefore, seems to correspond more closely than any other known natural polysaccharide to an idealized κ-carrageenan.

Water-soluble polysaccharides extracted from *E. cottonii*, *E. spinosum*, *E. isiforme*, *E. uncinatum*, *Furcellaria fastigiata*, *Gigartina canaliculata*, *G. chamissoi*, *G. atropurpurea*, *Ahnfeltia durvillaei*, *Gymnogongrus furcellatus*,

[190] R. A. Jones, E. J. Staples, and A. Penman, *J.C.S. Perkin II*, 1973, 1608.
[191] A. L. Stone, *Biopolymers*, 1972, **11**, 2625.
[192] N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J.C.S. Perkin I*, 1973, 2173.

Aghardhiella tenera, *Pachymenia hymantophora*, and *Gloiopeltis cervicornis* have been separated, where possible, into 3,6-anhydrogalactose-rich and -poor components (*i.e.* into χ- and λ-fractions).[193] Sixteen polysaccharides were investigated by direct analysis, by measurement of the sulphate eliminated to form additional 3,6-anhydro-D-galactose residues, and by determination of the proportion of the structure composed of alternately arranged D-galactose and 3,6-anhydro-D-galactose residues, before and after elimination of sulphate. The results are consistent with existing concepts of the structural chemistry of these polysaccharides; however, several new forms were noted, including an agarose sulphate and several new variants of λ-carrageenan. The structures of these polysaccharides were also studied by methylation.[194] Among structural variations noted in the χ- and ι-carrageenans were undersulphation on C-4 of the 3-linked residues and on C-6 of the 4-linked residues. A new type of polysaccharide was found in certain *Gigartina* species, the hypothetical, idealized form being named ξ-carrageenan; this is a polymer of D-galactose 2-sulphate in which the glycosidic linkages are $(1 \rightarrow 4)$ and $(1 \rightarrow 3)$, and probably arranged alternately with β- and α-configurations, respectively. 3,6-Di-*O*-methyl-D-galactose has been synthesized and shown to be identical with a product from the methylation analysis of polysaccharides related to ξ-carrageenan.[195] An oxidative hydrolysis method applied to polysaccharides containing 3,6-anhydro-D-galactose residues gave oligosaccharide sulphates in high yield; separation and characterization of these oligosaccharides enabled the positions of the sulphate groups to be established.[196] The presence was confirmed in κ-carrageenan of 3-linked D-galactose 4-sulphate residues as the major sulphate ester, with smaller amounts of 4-linked 3,6-anhydro-D-galactose 2-sulphate and 4-linked D-galactose 6-sulphate also present. Methylation analysis of a carrageenan fraction from *Gigartina skottsbergii* agreed qualitatively with previous suggestions for the polysaccharide's structure,[196a] except that 3-linked D-galactose 4-sulphate was shown to be present rather than the corresponding 2-sulphate.[197] For every ten D-galactose residues linked at C-3 there are, on average, six residues of 3,6-anhydro-D-galactose 2-sulphate linked at the C-4 position.

Miscellaneous Algal Polysaccharides.—Laminarin, alginic acid, 'fucans', and cellulose have been separated and characterized from extracts of *Himanthalia lorea*, *Bifurcaria bifurcata*, and *Padina pavonia*.[198] Fucans present in sequential extracts were shown to be composed of variable

[193] C. J. Lawson, D. A. Rees, D. J. Stancioff, and N. F. Stanley, *J.C.S. Perkin I*, 1973, 2177.
[194] A. Penman and D. A. Rees, *J.C.S. Perkin I*, 1973, 2182.
[195] A. Penman and D. A. Rees, *J.C.S. Perkin I*, 1973, 2188.
[196] A. Penman and D. A. Rees, *J.C.S. Perkin I*, 1973, 2191.
[196a] A. S. Cerezo, *J. Chem. Soc.* (*C*), 1967, 2491.
[197] A. S. Cerezo, *Carbohydrate Res.*, 1973, **26**, 335.
[198] A. J. Mian and E. Percival, *Carbohydrate Res.*, 1973, **26**, 133.

proportions of L-fucose, D-xylose, D-glucuronic acid, D-galactose (traces), and half-sulphate esters. Chromatography on ion-exchange cellulose gave fractions of highly sulphated materials with a high L-fucose content, polymers having a high content of D-glucuronic acid and a low content of sulphate, as well as polysaccharides intermediate between these two extremes. Methylation, periodate oxidation, and partial acidic hydrolysis revealed an essential similarity between the different L-fucose-containing polysaccharides from *B. bifurcata*.[199] A high L-fucose- and sulphate-containing polysaccharide was shown to possess (1 → 2)- and (1 → 3)-linked residues with sulphate groups at the C-4 position, and sulphate-free D-glucuronic acid and D-xylose residues on the periphery of the highly branched molecule. In a polymer with a high content of uronic acid, the L-fucose residues are similarly linked, and the D-xylose and most of the D-glucuronic acid residues are again present as end-groups and (1 → 4)-linked residues on the outside of the highly branched molecule. A g.l.c. study of the components of sargassa, a sulphated polysaccharide isolated from *Sargassum pallidum*, has indicated the presence of D-glucuronic acid, D-xylose, L-rhamnose, and L-fucose.[200] A recently developed solvolytic method [201] has been used to remove the sulphate groups from acid-labile polysaccharides of the red seaweed *Langia pacifica*.[202] Studies of the original and the desulphated polysaccharides by periodate oxidation [203] and by methylation [204] provided information on its structure, which consists of (1 → 3)-linked and some (1 → 4)-linked D-galactose units, and with D-xylose residues attached to the (1 → 4)-linked D-galactose units at the C-2 position.

A water-soluble, acid polysaccharide containing variable proportions of D-galactose, L-rhamnose, D-xylose, 4-*O*-methyl-D-galactose, and D-glucuronic acid, together with half-ester sulphate, has been isolated from caps and stalks of the unicellular green seaweed *Acetabularia crenulata*.[205] From partial desulphation, methylation, periodate oxidation, and partial acidic hydrolysis of the polysaccharide, it was established that (1 → 3)-linked D-galactose 4- and 6-sulphates, and (1 → 2)-linked L-rhamnose are features of the main chain. Residues of D-glucuronic acid, D-galactose, and L-rhamnose are present as end-groups, indicating the existence of a highly branched molecule with the uronic acid linked to both L-rhamnose and D-galactose residues. The acidic components released on partial acidic

[199] A. J. Mian and E. Percival, *Carbohydrate Res.*, 1973, **26**, 147.
[200] T. F. Solov'eva, V. A. Khomenko, and Y. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, 253.
[201] N. K. Kochetkov, A. I. Usov, and K. S. Adamyants, *Zhur. obshchei Khim.*, 1972, **42**, 1617.
[202] A. I. Usov, L. I. Miroshnikova, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1972, **42**, 945.
[203] A. I. Usov, L. I. Miroshnikova, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1972, **42**, 1623.
[204] N. K. Kochetkov, A. I. Usov, and L. I. Miroshnikova, *Zhur. obshchei Khim.*, 1972, **42**, 2309.
[205] E. Percival and B. Smestad, *Carbohydrate Res.*, 1972, **25**, 299.

hydrolysis of the polysaccharide from *Anatheca dentata* have been characterized as 4-*O*-(α-D-glucopyranosyluronic acid)-L-galactose, 4,6-*O*-(1-carboxyethylidene)-D-galactose, and 4-*O*-[4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl]-D-galactose.[206] Alkaline treatment of the polysaccharide revealed that only a small part of the sulphate is suitably located for the formation of 3,6-anhydro-rings, whereas periodate oxidation showed that the D-xylose residues are non-sulphated and either (1 → 4)- or (1 → 2)-linked and/or present as non-reducing end-groups.

The major acid polysaccharide from the mucilage of the fresh-water red alga of the genus *Batrachospermum* has been separated from a neutral polysaccharide and was found to contain D- and L-galactose, D-mannose, D-xylose, L-rhamnose, D-glucuronic acid, and two *O*-methyl sugars, which were characterized as 3-*O*-methyl-L-rhamnose (L-acofriose) and 3-*O*-methyl-D-galactose.[207] Two preponderant acidic oligosaccharides, obtained from partial acidic hydrolysates, were shown to contain galactose and D-glucuronic acid (1 : 1), suggesting a repeating sequence of these residues as the major structural feature of the polysaccharide. Investigation of the acetolysis products from a partially desulphated polysaccharide isolated from *Pachymenia carnosa* has led to the identification and characterization of the oligosaccharides (10)—(22).[208] Two polysaccharides were separated

α-D-Gal*p*-(1 → 3)-D-Gal
(10)

β-D-Gal*p*-(1 → 4)-D-Gal
(11)

2-*O*-Me-α-D-Gal*p*-(1 → 3)-D-Gal
(12)

D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal
(13)

D-Gal*p*-(1 → 3)-6-*O*-Me-D-Gal
(14)

6-*O*-Me-β-D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal
(15)

β-D-Gal*p*-(1 → 4)-α-D-Gal*p*-(1 → 3)-D-Gal
(16)

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Gal
(17)

[206] J. R. Nunn, H. Parolis, and I. Russell, *Carbohydrate Res.*, 1973, **29**, 281.
[207] J. R. Turvey and L. M. Griffiths, *Phytochemistry*, 1973, **12**, 2901.
[208] A. J. Farrant, J. R. Nunn, and H. Parolis, *Carbohydrate Res.*, 1972, **25**, 283.

β-D-Gal*p*-(1 → 4)-2-*O*-Me-α-D-Gal*p*-(1 → 3)-D-Gal

(18)

2-*O*-Me-α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Gal

(19)

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal

(20)

2-*O*-Me-α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-2-*O*-Me-D-Gal

(21)

β-D-Gal*p*-(1 → 4)-α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Gal*p*

(22)

by ion-exchange chromatography from the extracellular polysaccharides produced by *Anabaena flos-aquae*.[209] The neutral polysaccharide is composed mainly of D-glucose and minor amounts of D-xylose; D-glucose residues possibly constitute the polysaccharide core to which the D-xylose residues are attached. The acid polysaccharide is composed mainly of D-glucuronic acid and small proportions of D-xylose and D-ribose. The crystalline, microfibrillar structure of *Valonia ventricosa* cellulose has been studied by electron diffraction, which revealed no periodicity of amorphous to crystalline zones along the fibril axis.[210] Periodic chain-folding, as in natural cellulose, was considered to be unlikely.

A sulphated mannan has been isolated from the red seaweed *Nemalion vermiculare*, and the sulphate groups were found to be stable under conditions used for the elimination of such groups from sulphated galactans.[211] Desulphation with methanolic hydrogen chloride liberated a neutral mannan containing essentially linear chains of α-(1 → 3)-linked D-mannopyranose units. A xylan, having $[\alpha]_D = -83°$, was isolated by alkaline extraction of the insoluble residue of the alga and it appeared from methylation analysis to be a linear or slightly branched β-(1 → 4)- and β-(1 → 3)-linked polysaccharide. A water-soluble xylan isolated from *Chaetangium erinaceum* has been characterized as a linear molecule composed exclusively of β-(1 → 3)- and β-(1 → 4)-D-xylopyranose units (2 : 9) on the bases of methylation and partial acidic hydrolysis.[212] Evidence was obtained for the existence of regions of adjacent (1 → 4)-linkages interspersed with (1 → 3)-linkages, but none for the existence of contiguous (1 → 3)-linkages. The extracellular polysaccharide isolated from the cell-free medium of *Anacystis nidulans* is composed of glucose, galactose, and mannose, and production of the material depended on the age of the culture, the temperature of growth, and forms of nitrogen available.[213] By alkaline extraction

[209] W. S. Wang and R. G. Tischer, *Arch. Mikrobiol.*, 1973, **91**, 77.
[210] R. Lazaro and J. Chiaverina, *Cellulose Chem. Technol.*, 1973, **7**, 269.
[211] A. I. Usov, K. S. Adamyants, S. V. Yarotsky, A. A. Anoshina, and N. K. Kochetkov, *Carbohydrate Res.*, 1973, **26**, 282.
[212] J. R. Nunn, H. Parolis, and I. Russell, *Carbohydrate Res.*, 1973, **26**, 169.
[213] V. K. Sangar and P. R. Dugan, *Appl. Microbiol.*, 1972, **24**, 732.

of the cell walls of *Scendesmus obliquus*, a complex mixture of polysaccharides, composed of glucose, mannose, galactose, xylose, arabinose, ribose, and rhamnose, was solubilized.[214] Fractionation led to the isolation of a glucomannan.

Miscellaneous Protozoan Polysaccharides.—The unicellular protozoan *Prototheca zopfii* synthesizes a mixture of polysaccharides, the major component being a galactan with a branched structure having main chains of (1 → 6)-D-galactopyranose residues containing terminal D-galactofuranose residues.[215] A glycogen-type polysaccharide and a (1 → 4)-linked mannan are also produced. Four polysaccharide fractions, obtained by acidic and alkaline degradations of the purified cell walls of *Prototheca* sp. and *Chlorella* sp., were further hydrolysed and the resulting sugars were identified.[216] Five of the *Prototheca* sp. and two of the *Chlorella* sp. were indicated to have essentially similar polysaccharide compositions, which differ from those of *C. vulgaris* and *C. pyrenoidosa*, thus emphasizing the close affinity of *C. protothecoides* to the *Prototheca* sp. not shared by the other *Chlorella* sp. The storage-polysaccharide bodies in trophozoites of *Gregarina blaberae* contain an amylopectin having a chain length of nineteen D-glucose residues and with properties closely resembling the amylopectin from the coccidia *Eimeria tenella*.[217] A study of the fine structure of this polysaccharide, using a debranching enzyme from *Cytophaga*, showed that the unit-chain profile of the debranched polysaccharide is intermediate between those of a plant amylopectin and an animal glycogen, and is similar to that of the phytoglycogen of sweet corn.

[214] S. Kocurik, V. Novotna, and A. Grejtovsky, *Biologia*, 1973, **28**, 211.
[215] D. J. Manners, I. R. Pennie, and J. F. Ryley, *Carbohydrate Res.*, 1973, **29**, 63.
[216] M. V. Conte and R. S. Pore, *Arch. Mikrobiol.*, 1973, **92**, 227.
[217] C. Mercier, J. Schrevel, and J. R. Stark, *Comp. Biochem. Physiol.*, 1973, **44B**, 1001.

4
Microbial Polysaccharides

BY R. J. STURGEON

Bacterial Cell Walls and Membranes

Recent advances in the study of surface polysaccharides of bacterial cells have been reviewed, with sections on the site of peptidoglycan deposition in cocci, the role of autolytic enzymes in growth and division, the enzymes of peptidoglycan biosynthesis, and the synthesis of specialized cell-wall polymers such as lipopolysaccharides and teichoic acids.[1] The cellular and extracellular polysaccharides of both Gram-positive and Gram-negative bacteria have also been reviewed.[2]

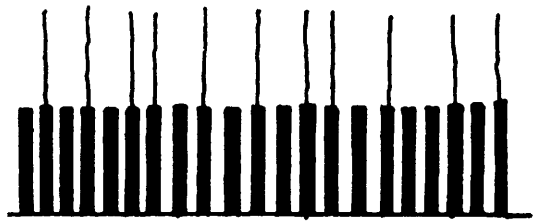
Teichoic Acids.—The teichoic acids have been discussed with respect to their location, constancy of occurrence and structure, immunogenicity, determination of the antigen–antibody reaction, specificity of the antibodies, action as group antigens, and immunobiological properties.[3] Taxonomic implications of teichoic and teichuronic acids have been reviewed.[4]

A stabilized L-form of *Streptococcus pyogenes* continued to synthesize a glycerol teichoic acid, which was found to be devoid of D-alanine and to have a shorter chain length than a corresponding polysaccharide isolated from *S. pyogenes*.[5] Otherwise, the glycerol teichoic acids from these organisms were found to be 1,3-phosphodiester-linked glycerol phosphate polymers substituted with D-glucose residues. Evidence was presented to show that most, if not all, of the glycerol teichoic acid in this streptococcus lies between the wall and the membrane. A possible need for the continued synthesis of a minute amount of glycerol teichoic acid by the L-form for survival was discussed in terms of the known functions of teichoic acids in bacteria. Antiserum against a strain of *Lactobacillus acidophilus* gave a reaction of identity with acid extracts of lactobacilli, some pediococci, leuconostocs, and *Staphylococcus aureus*.[6] The antigenic determinant isolated from a strain of *L. casei* was identified as a membrane glycerol teichoic acid. Purified membrane lipoteichoic acids from *Lactobacillus*

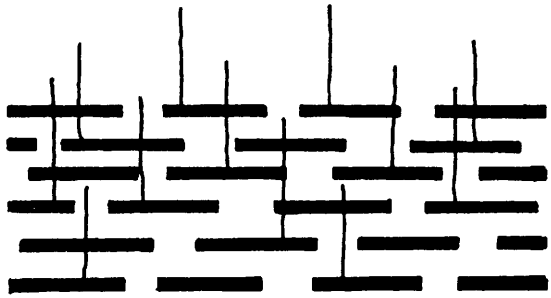
[1] L. Glaser, *Ann. Rev. Biochem.*, 1973, **42**, 91.
[2] 'Carbohydrates', ed. G. O. Aspinall, in 'MTP International Review of Science, Organic Chemistry Series One', Butterworths, London, 1973, Vol. 7.
[3] K. W. Knox and A. J. Wicken, *Bacteriol. Rev.*, 1973, **37**, 215.
[4] K. H. Schleifer and O. Kandler, *Bacteriol. Rev.*, 1972, **36**, 407.
[5] B. M. Slabyj and C. Panos, *J. Bacteriol.*, 1973, **114**, 934.
[6] M. E. Sharpe, J. H. Brock, K. W. Knox, and A. J. Wicken, *J. Gen. Microbiol.*, 1973, **74**, 119.

serological groups A, B, C, D, and F and streptococcal poly(glycerol phosphate) also reacted with the antiserum. The antigen–antibody reaction was partly inhibited by glycerol 1-phosphate and, together with the results of other inhibition studies, it was concluded that the antiserum is reacting with the common poly(glycerol phosphate) backbone of the membrane teichoic acid in the reacting strains. A large number of strains of *S. aureus* isolated from animal hosts have been examined for wall teichoic acids and other wall polymers by use of agar gel precipitation techniques.[7] The species was shown to be heterogeneous with regard to these constituents. A teichoic acid antigen isolated from a strain of *S. aureus* was shown to have a unique structure of poly(glycerol phosphate), with 2-acetamido-2-deoxy-D-glucose as the immunodominant substituent.[8] The molecular arrangement of teichoic acid in the cell wall of *Staphylococcus lactis* I3 has been studied by the analysis of purified, solubilized products obtained from digestion with a *Flavobacterium* enzyme.[9] The findings are consistent with two models for the distribution of peptidoglycan and teichoic acid in the wall: one in which the glycan chains lie radially and having 40% of these chains attached to the teichoic acid on an outside layer as in (1), or one in which the glycan chains lie parallel to the surface and with the teichoic acid fairly uniformly distributed throughout the wall as in (2). Two strains of *S. aureus* capable of adsorbing bacteriophages showed markedly reduced adsorbtion after pre-incubation with concanavalin A.[10] This effect was attributed to the specificity of the interaction of concanavalin A with sugars on the surface of the bacteria, and probably involves α-linked 2-acetamido-2-deoxy-D-glucosyl residues of the wall teichoic acids. This conclusion was strengthened by the failure of the lectin to effect the adsorption by *S. aureus* A1, which has been reported to contain β-linked hexosamine residues. It was suggested that these procedures might form the basis of a correlation between bacteriophage and serological types of staphylococci. Variation of the culture pH was found to effect changes not only in the amount, but also in the ester and bound-alanine contents, of teichoic acids in the walls of *Bacillus subtilis*.[11] The α-D-glucosylated teichoic acid component of the cell walls of *B. subtilis* has been isolated after chromatography on columns of concanavalin A–Sepharose.[12] The recovered polymer, prepared by affinity chromatography from cell-wall autolysates, had a higher sedimentation rate than teichoic acids obtained by conventional methods. Two poorly lytic mutants of *B. licheniformis*, containing no teichuronic acids in their walls, ceased to synthesize teichoic acid when grown in a limited supply of phosphate, but the amount of mucopeptide was greatly increased.[13] *X*-Ray photoelectron

[7] P. Oeding, *Acta Pathol. Microbiol. Scand.*, 1973, **81B**, 327.
[8] W. J. Reeder and R. D. Ekstedt, *Infection and Immunity*, 1973, **7**, 586.
[9] A. R. Archibald, J. Baddiley, and J. E. Heckels, *Nature New Biol.*, 1973, **241**, 29.
[10] A. R. Archibald and H. E. Coapes, *J. Gen. Microbiol.*, 1972, **73**, 581.
[11] D. C. Ellwood and D. W. Tempest, *J. Gen. Microbiol.*, 1973, **73**, 395.
[12] R. J. Doyle, D. C. Birdsell, and R. E. Young, *Prep. Biochem.*, 1973, **3**, 13.
[13] C. W. Forsberg, P. B. Wyrick, J. B. Ward, and H. J. Rogers, *J. Bacteriol.*, 1973, **113**, 969.



(1)



(2)

Possible models for arrangement of teichoic acid:
■, peptidoglycan; ——, teichoic acid

spectroscopy of Mg^{2+} ions bound to the cell walls of Gram-positive bacteria has been used to support the theory that, in the presence of esterified alanine residues in teichoic acids, a fraction of the Mg^{2+} ions is less strongly bound to the wall than in the alanine-free walls.[14] A relatively small, but significant, reduction in the equivalent porosity of the protoplast membrane of *B. megaterium*, but not in that of the wall of intact cells, was observed in the presence of 20 mmol l^{-1} magnesium chloride.[15] From a survey of the nature of the phosphorylated wall polymers in a number of micrococci, the presence of typical glycerol and ribitol teichoic acids having different sugar substituents was observed, in addition to atypical teichoic acids in which the repeating unit comprises glycerol phosphate and 2-acetamido-2-deoxy-D-deoxy-D-glucose 1-phosphate, and polymers of sugar phosphate.[16] Distinct chemo-groups could be recognized based on the composition and structure of the wall polymers. Dextrans from *Leuconostoc mesenteroides*, but not glycogen, amylose, or amylopectin, reacted with antisera to ribitol and glycerol teichoic acids from *Lactobacillus plantarum*; the antibodies are specific for the α-D-glucosidic residues in these teichoic acids.[17]

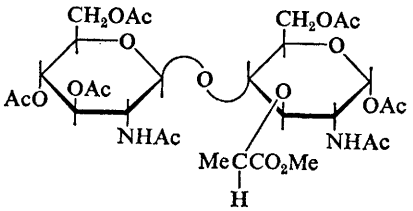
[14] J. Baddiley, C. Hancock, and P. M. A. Sherwood, *Nature*, 1973, **243**, 43.
[15] R. Scherrer and P. Gerhardt, *J. Bacteriol.*, 1973, **114**, 888.
[16] M. D. Partridge, A. L. Davison, and J. Baddiley, *J. Gen. Microbiol.*, 1973, **74**, 169.
[17] K. W. Knox and A. J. Wicken, *Arch. Oral Biol.*, 1972, **17**, 1491.

Lipoteichoic acids, virtually free from protein after chromatography, were isolated from membranes of Gram-positive bacteria.[18] It was thought unlikely that these polymers are covalently attached to either protein or nucleic acids, although these molecules profoundly influence their physical properties, especially with respect to micelle formation. No evidence was found for the occurrence of free, membrane teichoic acids in the organisms studied, and it was thought likely that all the membrane teichoic acid is present in the form of lipoteichoic acid. The highest yield of lipoteichoic acids from *L. fermenti* was obtained by extraction with hot phenol, yielding a product that contained very little protein, whereas a lipoteichoic acid–protein complex was isolated from aqueous extracts of the organism after partial removal of lipids.[19] The complex was shown to be a very effective immunogen; the immunogenicity was shown to relate to the protein content, although the specificity of the antibodies is directed against the teichoic acid components. The lipoteichoic acids isolated from both *L. fermenti* and *L. casei* were immunogenic and cross-reacted serologically.[20] Electron-microscopic studies of ferritin-coupled anti-lipoteichoic acid antibodies (used for the detection of absorbed antibodies) indicated that both organisms have at least part of their lipoteichoic acid exposed near enough to the surface of the cell for reaction with the antibody. It was established that the lipoteichoic acid is mainly located in the cell membrane. Differences in the *in situ* serological behaviour of the polymers in the two organisms were explained on the basis of a model involving a variable depth of penetration of the membrane-located teichoic acid chains into the polysaccharide–peptidoglycan network of the cell wall. A streptococcus having a group antigen Z_3 and a type III antigen was shown to have a much higher carbohydrate content (33%) in the cell walls than that (12%) of a mutant strain lacking D-galactose.[21] Extraction with phenol solubilized a serologically active component from the mutant strain which reacted with anti-group (Z_3) serum; this component contained glycolipid, glycerol, phosphate, and alanine, and is thought to be a lipoteichoic acid. Pneumococcal Forssman antigen (a substance that cross-reacts with the Forssman series of mammalian surface antigens) has been shown to contain choline in a lipoteichoic acid on the basis of tritium labelling, hemolysis-inhibition assays, and binding studies with a myeloma protein.[22] Freezing and thawing released a non-sedimentable lipoprotein, which is active in the biosynthesis of wall teichoic acids, and an undecaprenol phosphate intermediate in the synthesis of the D-glucose-containing polymer.[23] Incorporation of [^{14}C]mevalonic acid, predominantly into

[18] J. Coley, M. Duckworth, and J. Baddiley, *J. Gen. Microbiol.*, 1973, **73**, 587.
[19] A. J. Wicken, J. W. Gibbens, and K. W. Knox, *J. Bacteriol.*, 1973, **113**, 365.
[20] D. van Driel, A. J. Wicken, M. R. Dickson, and K. W. Knox, *J. Ultrastruct. Res.*, 1973, **43**, 483.
[21] J. H. J. Huis In'T Veld, *J. Microbiol. Serol.*, 1972, **38**, 631.
[22] E. B. Briles and A. Tomasz, *J. Biol. Chem.*, 1973, **248**, 6394.
[23] I. C. Hancock and J. Baddiley, *F.E.B.S. Letters*, 1973, **34**, 15.

C_{55}-prenol, has made it possible to determine the distribution of [^{14}C]-prenol between all its derivatives in the biosynthesis of the walls in growing cells of *L. plantarum*.[24] In log-phase cells, 12% was found as a peptidoglycan precursor and 28% as a glycophosphoprenol (containing rhamnose, glucose, galactose, and ribitol phosphate), and these components may be involved in the syntheses of peptidoglycan and teichoic acid. The role of isoprenoid intermediates in the biosynthesis of bacterial cell walls has been reviewed.[25]

Peptidoglycans.—The types of peptidoglycan in bacterial cell walls and their taxonomic implications have been reviewed.[4] The immunochemistry of a peptidoglycan-precursor pentapeptide has been studied using the pentapeptide Ala-γ-D-Glu-Lys-D-Ala-D-Ala covalently linked to a random polypeptide.[25a] This conjugate evoked an antibody response in immunized rabbits. The immunodominant region was shown by radioimmunoassay to comprise the carboxy-terminus of the pentapeptide *X*-Lys-D-Ala-D-Ala. The glycan chain length of the peptidoglycans was measured after reduction with sodium borotritide and isolation of the resulting muraminitol, which indicates the length of the synthesized chains, and glucosaminitol, which measures the length of the chains after rupture by *endo*-β-2-acetamido-2-deoxyglucosidases.[26] A synthesis of the repeating disaccharide unit of the glycan moiety of bacterial cell walls has been described.[27] The product (3)



(3)

proved to be identical with that obtained from the glycan following digestion with lysozyme and acetylation. Analysis of the outer membrane and other layers of the cell envelope of an *Acinetobacter* sp. showed that outside the plasma membrane and periplasmic space the envelope is composed of a peptidoglycan, an intermediate layer, a lipopolysaccharide-containing outer membrane, and finally an ordered array of protein subunits.[28] In seventeen strains of coryneform bacteria, 2,4-diaminobutyric acid was found to be a component of the peptidoglycan.[29] A detailed analysis

[24] K. J. I. Thorne, *J. Bacteriol.*, 1973, **116**, 235.
[25] J. Baddiley, in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[25a] A. R. Zeiger and P. H. Maurer, *Biochemistry*, 1973, **12**, 3387.
[26] J. B. Ward, *Biochem. J.*, 1973, **133**, 395.
[27] C. Merser and P. Sinaÿ, *Tetrahedron Letters*, 1973, 1029.
[28] K. J. I. Thorne, M. J. Thornley, and A. M. Glauert, *J. Bacteriol.*, 1973, **116**, 410.
[29] F. Fiedler and O. Kandler, *Arch. Mikrobiol.*, 1973, **89**, 51.

showed that fifteen of the strains contain a peptidoglycan having the same amino-acid sequence as that found in *Corynebacterium insidiosum*. In the latter case, D-2,4-diaminobutyric acid serves as an interpeptide bridge between D-glutamic acid and *C*-terminal D-alanine residues. The two remaining strains differed by lacking D-2,4-diaminobutyric acid, as only the L-isomer was found. An unusual type of bacterial cell wall, in which teichoic acid is replaced by a protein of high molecular weight and the peptidoglycan fraction contains 2-amino-2-deoxy-D-galactose and an excess of 2-amino-2-deoxy-D-glucose, has been identified in a mutant of *B. subtilis*.[30] Analyses involving dinitrophenylation have revealed the occurrence of significant proportions of 2-amino-2-deoxy-D-glucose residues in the peptidoglycan components of *B. cereus*, *B. subtilis*, and *B. megaterium*.[31] A close correlation was demonstrated between the content of the non-*N*-acetylated amino-sugar residues and the resistance of the walls to lysozyme. The lysozyme-resistant cell walls and peptidoglycans were converted into a lysozyme-sensitive form by *N*-acetylation. Treatment of the peptidoglycan from *B. cereus* with *N*-acetylmuramyl-L-alanine amidase, followed by digestion with lysozyme, released three oligosaccharides (4)—(6) containing 2-amino-2-deoxy-D-glucose residues.[32]

D-GlcNH_2-Mur*N*Ac-D-Glc*N*Ac-Mur*N*Ac

(4)

D-GlcNH_2-Mur*N*Ac-D-GlcNH_2-Mur*N*Ac-D-Glc*N*Ac-Mur*N*Ac

(5)

D-Glc*N*Ac-Mur*N*Ac-D-GlcNH_2-Mur*N*Ac-D-Glc*N*Ac-Mur*N*Ac

(6)

Lysozyme was unable to hydrolyse oligosaccharides (4) and (5), but cleaved oligosaccharide (6) to yield 4-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-*N*-acetylmuramic acid and oligosaccharide (4). The effect of an insufficient supply of phosphate on the morphology and cell-wall composition of *B. licheniformis* and its phosphoglucomutase-deficient mutants was to produce greatly increased proportions of mucopeptide, which formed irregular spheres under these conditions.[13] By analysis of autolytic digests of the log-phase cell walls of *B. psychrophilus*, it was shown that the amino-acid sequence of the peptide sub-unit in the peptidoglycan consists of muramyl-L-alanyl-γ-D-glutamyl-L-lysyl-D-alanine, and that the linkage between adjacent peptides is supplied by a second D-glutamic acid residue bound to the ε-amino-group of lysine and the carboxy-group of D-alanine.[33] Walls isolated from the partial septa of *B. subtilis* were more

[30] M. Leduc, J. Van Heijenoort, M. Kaminski, and J. Szulmajster, *European J. Biochem.*, 1973, **37**, 389.
[31] H. Hayashi, Y. Araki, and E. Ito, *J. Bacteriol.*, 1973, **113**, 592.
[32] H. Hayashi, K. Amano, Y. Araki, and E. Ito, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 641.
[33] G. K. Best and S. J. Mattingly, *J. Bacteriol.*, 1973, **115**, 221.

sensitive to digestion by *B. subtilis* autolytic amidase than end walls from hemispherical caps, suggesting that the end walls are modified to an amidase-resistant form after synthesis.[34] Alteration of the spores of *Clostridium perfringens* by either heat or alkali apparently inactivated the normal lytic system responsible for cortical degradation during germination.[35] Germination recommenced after the addition of either lysozyme or an initiator protein produced by *C. perfringens*. Appendages removed from the spores of *C. taeniosporum* are composed of seventeen amino-acids; 2-acetamido-2-deoxy-D-glucose is the most abundant sugar present, but muramic acid is absent.[36]

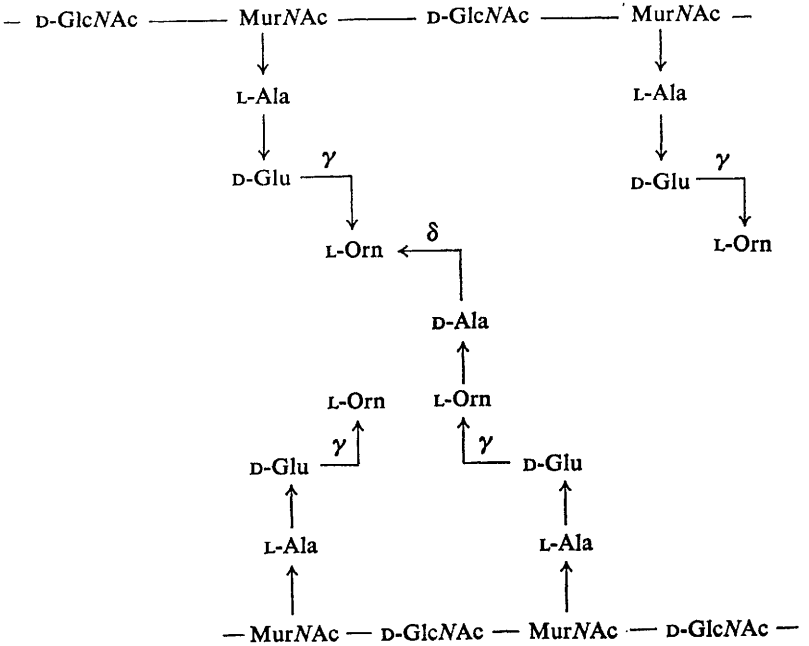
A lytic enzyme induced by infection of *Escherichia coli* with bacteriophage T7 was shown to be an *N*-acetylmuramyl-L-alanine amidase by analysis of the products of the enzyme's action on a purified *E. coli* peptidoglycan containing a lipoprotein covalently attached to diaminopimelic acid.[37] The surface area per repeating peptidoglycan unit (per molecule of diaminopimelate) for the cell envelopes of *E. coli* strains was found to be 1.3 nm^2.[38] Using this value and other previously determined properties of the peptidoglycans, a three-dimensional model was proposed. The model specified a monomolecular layer in which disaccharide units are each 1.03 nm long and the polysaccharide chains, all parallel, are 1.25 nm apart. The cross-linking peptide side-chains have the same atomic co-ordinates and are arranged above or below the polysaccharide chains. Using radio-isotope techniques to determine the immunological cross-reactions in separated membranes of *E. coli*, it was demonstrated that the lipoprotein attached to the peptidoglycan extends only into the outer membrane, where the free form (*i.e.* lipoprotein not attached to the peptidoglycan) is also localized.[39] The peptidoglycan sacculi of *E. coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* are considered to be either part of the penetration barrier or responsible for holding together the structure of the outer membrane.[40] Both D-alanyl-D-alanine and UDP-*N*-acetyl-muramylpentapeptide were synthesized in decreasing amounts when a temperature-sensitive mutant of *E. coli* was grown at two different temperatures.[41] The lower rate of synthesis was accompanied, in one case, by an accumulation of UDP-*N*-acetylmuramyltripeptide. The lipid intermediate involved in the synthesis of peptidoglycan in *E. coli* has been identified as a C_{55}-isoprenylpyrophosphoryl-*N*-acetylmuramylpentapeptide-2-acetamido-2-deoxy-D-glucoside.[42] Evidence for the *in vivo* biosynthesis

[34] D. P. Fan and B. E. Beckman, *J. Bacteriol.*, 1973, **114**, 790.
[35] C. L. Duncan, R. G. Labbe, and R. R. Reich, *J. Bacteriol.*, 1972, **109**, 550.
[36] D. P. Yolton, R. N. Huettel, D. K. Simpson, and L. J. Rode, *J. Bacteriol.*, 1972, **109**, 881.
[37] M. Inouye, N. Arnheim, and R. Sternglanz, *J. Biol. Chem.*, 1973, **248**, 7247.
[38] V. Braun, H. Gnirke, U. Henning, and K. Rehn, *J. Bacteriol.*, 1973, **114**, 1264.
[39] V. Bosch and V. Braun, *F.E.B.S. Letters*, 1973, **34**, 307.
[40] L. G. Burman, K. Nordström, and G. D. Bloom, *J. Bacteriol.*, 1972, **112**, 1364.
[41] E. J. J. Lugtenberg and A. van Schijndel-van Dam, *J. Bacteriol.*, 1973, **113**, 96.
[42] J. N. Umbriet and J. L. Strominger, *J. Bacteriol.*, 1972, **112**, 1306.

of the peptidoglycan-lipoprotein in the outer layer of *E. coli* indicated that the lipoprotein is not randomly incorporated into pre-existing peptidoglycan, but rather into newly made, less-cross-linked peptidoglycan.[43] The quantitative chemical composition of the peptidoglycan isolated from intact cells of *Listeria monocytogenes* showed the presence of L-rhamnose, with 2-amino-2-deoxy-D-glucose in very high proportion compared with the amino-acids, muramic acid, or diaminopimelic acid.[44] Autolysis of *Lactobacillus fermenti* was accompanied by the release of reducing groups, amino-sugars, and free amino-groups in a manner suggesting that the system is a mixture of several enzymes.[45] In *Lactobacillus acidophilus*, the turnover of cell-wall peptidoglycan appeared to be closely associated with processes related to wall- (and surface-) enlargement, but not to those involved in wall-thickening.[46] The disaccharide 6-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosyluronic acid)-D-glucose has been isolated and characterized from an acidic, D-glucose-containing polysaccharide of *Micrococcus lysodeikticus*.[47] Rabbit antisera capable of precipitating the polysaccharide were prepared and from the specificity of the antigen–antibody reaction, as studied by quantitative precipitation-inhibition assay, it was concluded that the antigenic determinants of the polysaccharide contain α-linked D-glucopyranosyl and β-linked 2-acetamido-2-deoxy-D-mannopyranosyluronic acid structures.[48] The influence of the dye Biebrich Scarlet upon the lysis kinetics of *M. lysodeikticus* by a number of lysozymes has been reported.[49] Depleted membranes, containing 6—12% carbohydrate, have been prepared by protoplast lysis and various washing procedures from *M. lysodeikticus*; different patterns of carbohydrate-containing material were revealed by gel electrophoresis.[50] Peptidoglycan preparations from *Neisseria perflava*, *Moraxella glucidolytica*, and *Proteus vulgaris* were found to possess an adjuvant activity for the late hypersensitivity reactions.[51] The major disaccharide unit of the mucopeptide portion of the cell wall of *Nocardia asteroides* is 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-glycolylmuramic acid, thus illustrating the close relationship of this mucopeptide with those previously isolated from *N. kirovani* and the cell walls of mycobacterial species.[52] Synthesis of the wall peptidoglycan of *Pediococcus cerevisiae* continued under conditions that were inadequate to support growth.[53] Electron-

[43] V. Braun and V. Bosch, *F.E.B.S. Letters*, 1973, **34**, 302.
[44] K. K. Srivastava and I. H. Siddiqui, *Infection and Immunity*, 1973, **7**, 700.
[45] H. Y. Neujahr and I. M. Logardt, *Biochemistry*, 1973, **12**, 2578.
[46] D. Boothby, L. Daneo-Moore, M. L. Higgins, J. Coyette, and G. D. Shockman, *J. Biol. Chem.*, 1973, **248**, 2161.
[47] S. Hase, Y. Tsuji, and Y. Matsushima, *J. Biochem. (Japan)*, 1972, **72**, 1549.
[48] M. Torii, K. Sakakibara, S. Iida, K. Kobayashi, S. Hase, and Y. Matsushima, *Biken J.*, 1973, **16**, 11.
[49] J. S. Blancard, M. Allary, and P. Jollès, *Biochimie*, 1972, **54**, 1375.
[50] S. F. Estrugo, J. Coll, J. A. Leal, and E. Muñoz, *Biochim. Biophys. Acta*, 1973, **311**, 153.
[51] C. Nauciel, J. Fleck, J. P. Martin, and M. Mock, *Compt. rend.*, 1973, **276**, *D*, 3499.
[52] I. Azuma, F. Kanetsuna, Y. Tanaka, M. Mera, Y. Yanagihara, I. Mifuchi, and Y. Yamamura, *Jap. J. Microbiol.*, 1973, **17**, 154.
[53] B. J. Wilkinson and P. J. White, *J. Bacteriol.*, 1973, **116**, 507.

microscopic examination of cells of *Spirochaeta stenostrepta* treated with either lysozyme or penicillin showed that the peptidoglycan is present as a thin, electron-dense layer adjacent and external to the cytoplasmic membrane.[54] The glycans isolated from *S. stenostrepta* and *S. litoralis* were shown to contain 2-amino-2-deoxy-D-glucose, muramic acid, D-glutamic acid, L-ornithine, and D- and L-alanine, with nearly 50% of the L-ornithine residues involved in peptide cross-linkages; these data indicate that the spirochaetal peptidoglycan is similar to that of many Gram-negative bacteria, excepting that L-ornithine occurs instead of either L-lysine or diaminopimelic acid. The primary structure of the peptidoglycan of *S. stenostrepta* was elucidated and a typical fragment (7) is illustrated.[55]



(7)

The non-amidated disaccharide tetrapeptide 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramyl-L-alanyl-γ-D-glutamyl-(L)-*meso*-diaminopimelyl-(L)-D-alanine, the corresponding disaccharide-free tetrapeptide, and the tripeptide L-alanyl-γ-D-glutamyl-(L)-*meso*-diaminopimelic acid exhibited very similar properties as acceptors for transpeptidation of the wall peptidoglycan of *Streptomyces* R39.[56] Amidation of the

[54] R. Joseph, S. C. Holt, and E. Canale-Parola, *J. Bacteriol.*, 1973, **115**, 426.
[55] K. H. Schleifer and R. Joseph, *F.E.B.S. Letters*, 1973, **36**, 83.
[56] J.-M. Ghuysen, M. Leyh-Bouille, J. N. Campbell, R. Moreno, J.-M. Frére, C. Duez, M. Nieto, and H. R. Perkins, *Biochemistry*, 1973, **12**, 1243.

(D)-carboxy-group of *meso*-diaminopimelic acid prevented recognition of the peptide by the enzyme. Control mechanisms were recognized to include inhibition by excesses of acceptor and transpeptidated product and the sensitivity of the transpeptidated product to hydrolysis by the carboxypeptidase activity of the enzyme. From a study of whether wall-growth in Gram-negative bacteria occurs diffusely or at specific growth-points associated with the site of division, it was concluded that although the site of spheroplast emergence from penicillin-treated, Gram-negative bacilli in conditions of suitably high osmoregularity is at the incipient division point, this is not the unique site for the synthesis of mucopeptides in these organisms.[57] The peptide moiety of UDP-acetylmuramylpentapeptide has been shown to be synthesized in a reaction sequence involving five enzymes.[58] Data obtained from the association constants for bacitracin, Mg^{2+}, and C_{55}-isoprenyl pyrophosphate have indicated that both hydrophobic and polar interactions are involved in complex formation.[59] Reasonable correlation was found between the binding constants for various bacitracin peptides and their antibiotic activity.

Lipopolysaccharides.—An improved method for isolating the *Limulus* extract used in the determination of lipopolysaccharides has been reported.[60] The quantitative composition of sugars in the endotoxins of a number of strains of asporulating, Gram-negative, anaerobic rods has been determined.[61] The polysaccharide fractions isolated from all the endotoxins contained heptoses and, in the majority of cases, 2-keto-3-deoxyoctonic acid.[62] Termination of the tolerance to human γ-globulin in mice by bacterial lipopolysaccharides has been demonstrated.[63] The chemical compositions of myxobacterial lipopolysaccharides were shown to be analogous to those of other Gram-negative bacteria.[64] Many strains possess a polymer containing ribose, mannose, and galactose, as well as one or both of the amino-sugars 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose. Unlike most eubacterial lipopolysaccharides, heptose was either absent or present in very small proportions in myxobacterial materials. The chemical similarity of the lipopolysaccharides to analogous polymers from other Gram-negative bacteria confirmed the belief (from cytological studies) that the cell-wall myxobacteria resemble typical Gram-negative prokaryotes. The lipopolysaccharides isolated from four different strains of *Bordetella pertussis* all contained heptose, hexose, and 2-amino-2-deoxyhexoses.[65] Fractions

[57] D. Greenwood and F. O'Grady, *Naturwiss.*, 1973, **60**, 435.
[58] E. Ito and J. L. Strominger, *J. Biol. Chem.*, 1973, **248**, 3131.
[59] D. R. Storm and J. L. Strominger, *J. Biol. Chem.*, 1973, **248**, 3940.
[60] P. A. Ward and J. H. Hill, *Proc. Soc. Exp. Biol. Med.*, 1972, **141**, 898.
[61] W. Zawisza-Zenkteler and F. Meisel-Mikolacjzyk, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1973, **21**, 255.
[62] B. Kedzierska, F. Meisel-Mikolajczyk, and E. Mikulaszek, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1973, **21**, 187.
[63] J. M. Chiller and W. O. Weigle, *J. Exp. Med.*, 1973, **137**, 740.
[64] I. W. Sutherland and M. L. Smith, *J. Gen. Microbiol.*, 1973, **74**, 259.
[65] M. A. Aprile and A. C. Wardlaw, *Canad. J. Microbiol.*, 1973, **19**, 231.

of an endotoxin nature were obtained from four strains of *Eggerthella convexa* and were shown to contain variable quantities of hexose, amino-sugars, protein, and lipids; two of the strains contained uronic acid material and L-rhamnose, in addition to D-galactose, D-glucose, D-mannose, and D-ribose.[66] The ability of bacterial lipopolysaccharides from *Escherichia coli* and *Salmonella typhimurium* to induce lymphocyte mitogenesis and to act as an adjuvant of antibody formation was ascribed to the lipid-A region of the molecule.[67] Removal of the ester-linked fatty acid by alkaline hydrolysis abrogated the B-cell mitogenic stimulus obtained with the intact molecule. Lipopolysaccharides on the cell surface of *E. coli* have been shown to prevent the penetration of lysozyme and certain drugs of low molecular weight into the cell.[68] The absence of either phosphate or D-glucose residues in the polysaccharide portion of the lipopolysaccharide was closely related to the sensitivity of the cells to lysozyme. Agglutination studies have indicated that when *E. coli* K12 strain is crossed with an *E. coli* O8 donor, the recombinants synthesized O8-specific units and attached them to the K12 lipopolysaccharide core.[69] Since mutants of *E. coli* and *S. typhimurium*, in which the lipopolysaccharide layer does not contain residues beyond the 2-keto-3-deoxyoctonate core, are inhibited by medium- but not long-chain fatty acids, it has been suggested that the intact lipopolysaccharide layer of Gram-negative organisms screens the cells against these fatty acids by preventing their accumulation at the inner-cell membrane.[70] Although the D-galactose content of the lipopolysaccharide from *E. coli* cells was not appreciably reduced when the membrane-phospholipid was altered with respect to the chain length and position and configuration of the ethylenic bond of the fatty acids, reaction in the presence of UDP-D-galactose:lipopolysaccharide galactotransferase was found to be sensitive to changes in the fatty-acid structure of the phospholipid.[71] By use of lipopolysaccharides from *E. coli* mutants variously affected in lipopolysaccharide structure, correlations were established between the degree of defectiveness of the polysaccharide and its ability to adsorb bacteriophage C21; the adsorption was greatly decreased by the presence of small amounts of D-galactose or L-rhamnose in the lipopolysaccharide.[72] The phage-receptor is contained in the inner core of the K12 lipopolysaccharide, but 2-keto-3-deoxyoctonate is not an essential part of the phage-receptor. Investigation of the water-soluble products resulting from degradation of the lipopolysaccharide of *E. coli* O8 with

[66] F. Meisel-Mikolajczyk and A. Dworczyński, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1973, **21**, 193.
[67] J. M. Chiller, B. J. Skidmore, D. C. Morrison, and W. O. Weigle, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2129.
[68] S. Tamaki and M. Matsuhashi, *J. Bacteriol.*, 1973, **114**, 453.
[69] G. Schmidt, *J. Gen. Microbiol.*, 1973, **77**, 151.
[70] C. W. Sheu and E. Freese, *J. Bacteriol.*, 1973, **115**, 869.
[71] I. F. Beacham and D. F. Silbert, *J. Biol. Chem.*, 1973, **248**, 5310.
[72] A. M. C. Rapin and H. Mayer, *Experientia*, 1973, **29**, 756.

coliphage Ω8 showed that the polysaccharide is cleaved into the trisaccharide repeating unit *O*-α-D-mannosyl-(1 → 2)-*O*-α-D-mannosyl-(1 → 2)-D-mannose, and di-, tri-, and tetra-mers thereof.[73] Coliphage Ω8 also cleaved the lipopolysaccharides of *E. coli* O93 and *Klebsiella* O5 in the same fashion. These lipopolysaccharides were also receptors of coliphage Ω8, which is adsorbed on the respective bacteria, and inhibited the infection of *E. coli* O8 with the coliphage. It was also demonstrated that polysaccharides susceptible to the enzymic action of coliphage Ω8 cross-reacted serologically with the polysaccharide of *E. coli* O8. After incubation of a cell-wall particulate fraction of *E. coli* cells grown on tritiated 2-amino-2-deoxy-D-glucose with UDP-D-[^{14}C]galactose, under conditions designed to measure the galactosyltransferase activity, the isolated lipopolysaccharide–protein complex contained all of the D-[^{14}C]galactose.[74] The lipopolysaccharide was shown to occur exclusively in the outer cell-envelope membrane in the form of a protein complex.

Four forms of lipopolysaccharide containing *O*-methyl-D-glucoses from *Mycobacterium phlei* have been shown to contain from nil to three moles of ester succinate.[75] Each form was also acylated with acetate, propionate, isobutyrate, and octanoate, and eight positions of acylation have been ascertained by replacement of the ester groups with methyl groups. Lipopolysaccharides containing 6-*O*-methyl-D-glucose were demonstrated to contain isotopically labelled acyl groups when cells of *M. phlei* were incubated with ^{14}C-labelled, short-chain fatty acids.[76] The pattern of labelling of polysaccharides containing different acyl groups, the effects of different conditions on labelling patterns, and the kinetics of the turnover of ^{14}C-labelled acyl groups were studied. Six of the positions were shown to be acylated specifically with the monobasic acids, and two others specifically with succinyl groups.[77] A further site of succinylation was noted, thereby accounting for nine positions of acylation in one of the polysaccharides. The results of methyl replacement and Smith degradation indicated that the monobasic acids esterify positions 4 and 6 of the 3-*O*-methyl-D-glucose unit and position 6 of each of three adjacent D-glucose residues at the same end of the chain. An additional, neutral, acyl group, probably as octanoate, esterifies position 3 of glyceric acid. Propionate was located at two of the positions near the 3-*O*-methyl-D-glucose end of the polysaccharide, indicating that there is some heterogeneity in the placement of monobasic acids at these sites, since only one residue of propionate is normally present. The non-random distribution of monobasic and dibasic acids supports the concept that a defined placement of esters is in some way related to the biological function of the lipopolysaccharide.

[73] K. Reske, B. Wallenfels, and K. Jann, *European J. Biochem.*, 1973, **36**, 167.
[74] M. C. Wu and E. C. Heath, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2572.
[75] G. R. Gray and C. E. Ballou, *J. Biol. Chem.*, 1972, **247**, 8129.
[76] K. Narumi, J. M. Keller, and C. E. Ballou, *Biochem. J.*, 1973, **132**, 329.
[77] W. L. Smith and C. E. Ballou, *J. Biol. Chem.*, 1973, **248**, 7118.

A particulate enzyme preparation obtained from *M. phlei* cells was shown to be a polysaccharide:acyl coenzyme A acyltransferase involved in the biosynthesis of the polysaccharide, since it catalysed the transfer of acetyl, propionyl, isobutyryl, octanoyl, and succinyl groups to the polysaccharide.[78] Analysis of the product obtained in the enzymic reaction between [^{14}C]acetyl-coenzyme A and the methylated D-glucose polysaccharide provided evidence that at least two of the sites of acetylation occur in a tetrasaccharide unit at the non-reducing end of the polysaccharide; other incorporation studies indicated that the sites of succinylation are located elsewhere in the polysaccharide.

A lipopolysaccharide with pyocin R-receptor activity, isolated from *Pseudomonas aeruginosa*, has been dissociated and fractionated into a fraction rich in amino-sugars having no receptor activity and lipopolysaccharide subunits having receptor activity.[79] Analysis of the reassociated lipopolysaccharide, after removal of lipid-A, revealed the presence of D-glucose, L-rhamnose, heptose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, 2-amino-2,6-dideoxy-L-galactose, 2-amino-2,6-dideoxy-D-glucose, and a 2-keto-3-deoxy-sugar acid. The lipid-A fraction contained only D-glucose, L-rhamnose, heptose, 2-amino-2-deoxy-D-galactose, the 2-keto-3-deoxy-sugar acid, and phosphate. All the lipopolysaccharides isolated from fifteen strains of *P. aeruginosa* were found to contain heptose, D-glucose, L-rhamnose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, 2-keto-3-deoxyoctonic acid, and alanine.[80] Their composition and structure suggested the existence of a common region of low molecular weight in all the polysaccharides. The strains were classified by their chemistry and that of their degradation products into twelve chemo-groups, nine of which contained one serotype and three of which contained two serotypes. The serological specificity is limited to the regions of high molecular weight, possibly corresponding to the side-chains. The possibility that proteins may be associated with the terminal stages of lipopolysaccharide synthesis was suggested from a comparison of the proteins released from the cell envelopes of *P. aeruginosa* on exposure to H_4edta and extraction with DMF.[81] Some unusual features noted in the lipopolysaccharide composition of a number of *Pseudomonas* sp. included the occurrence of non-phosphorylated heptose residues (*P. diminuta*), the presence of phosphorylated D-glucose residues and aspartic acid (*P. pavonacea*), the probable absence of 2-keto-3-deoxyoctonic acid and the presence of acid-labile D-galactose residues (*P. rubescens*), and the virtual absence of D-glucose residues (*P. stutzeri*).[82] The amino-sugars 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and 2-amino-

[78] K. K. Tung and C. E. Ballou, *J. Biol. Chem.*, 1973, **248**, 7126.
[79] K. Ikeda and F. Egami, *J. Gen. Appl. Microbiol.*, 1973, **19**, 115.
[80] I. R. Chester, P. M. Meadow, and T. L. Pitt, *J. Gen. Microbiol.*, 1973, **78**, 305.
[81] J. D. Stinnett, H. E. Gilleland, and R. G. Eagon, *J. Bacteriol.*, 1973, **114**, 399.
[82] S. G. Wilkinson, L. Galbraith, and G. A. Lightfoot, *European J. Biochem.*, 1973, **33**, 158.

2,6-dideoxy-D-glucose occur only in the polysaccharides from *P. stutzeri* and *P. syncyanea*.

In addition to 3-*O*-methyl-L-xylose, lipopolysaccharides isolated from *Rhodopseudomonas viridis* have been found to contain 3-*O*-methyl-D-mannose.[83] Two strains of *R. palustris* were shown to contain 4-*O*-methyl-D-xylose and 6-deoxy-3-*O*-methyl-D-talose (D-acovenose). A number of strains of *R. palustris* have been classified into three distinct chemotypes on the basis of their carbohydrate composition.[84]

The chemical composition of polysaccharide fractions isolated from *Salmonella dahlem* by two different methods indicated that a system employing Cetavlon gave fractions containing the maximum amounts of sialic acid.[85] Further structural studies on the lipopolysaccharides from *S. kentucky* and *S. newport* have been reported, with slight modifications to the structures previously proposed.[86] Lipopolysaccharides isolated from different mutant strains of *S. minnesota* and *Escherichia coli* have been employed to investigate which part of the molecule exerts mitogenic effects on lymphocytes obtained from the bone marrow of mice.[87] Within the structure of the lipopolysaccharide, consisting of lipid A, core polysaccharide, and O-antigen, the lipid A was found to be the mitogenic part. Removal of the fatty acids from this part of the molecule destroyed the mitogenic effect. The cell-wall lipopolysaccharides of *S. minnesota*, *S. typhimurium*, and *Shigella flexneri* appeared in the electron microscope as long, slender filaments having a molecular weight of several millions.[88] Such molecules are comprised of subunits held together by low-energy bonds of chelating and hydrophobic nature due to the lipid part of the molecule. Studies of dissociation and reassociation of the lipopolysaccharide subunits showed the existence of a cylindrical, intermediate 'unit structure' having a molecular weight of 5×10^5. The arrangement of subunits in this structure seems to be of a micellar type, with the lipids located in the central part of the structure. The ability of the lipopolysaccharide of *S. typhimurium* to remove respiratory control of isolated rat-liver mitochondria could be used more efficiently to quantify the relative toxicities of these molecules than could determinations of LD_{50} for mice.[89] Counter-current distribution in an aqueous two-polymer phase system has been used in the characterization of mutants of *S. typhimurium*; the results demonstrated the great influence exerted by long-chain polysaccharides on the physicochemical properties of the

[83] J. Weckesser, H. Mayer, and I. Fromme, *Biochem. J.*, 1973, **135**, 293.
[84] J. Weckesser, G. Drews, I. Fromme, and H. Mayer, *Arch. Mikrobiol.*, 1973, **92**, 123.
[85] B. Kedzierska and E. Mikulaszek, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1973, **21**, 181.
[86] C. Hellerqvist, J. Hoffman, A. A. Lindberg, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 3282.
[87] J. Andersson, F. Melchers, C. Galanos, and O. Lüderitz, *J. Exp. Med.*, 1973, **137**, 943.
[88] E. Hannecart-Pokorni, D. Dekegel, and F. Depuydt, *European J. Biochem.*, 1973, **38**, 6.
[89] G. G. Greer, N. A. Epps, and W. J. Vail, *J. Infect. Dis.*, 1973, **127**, 551.

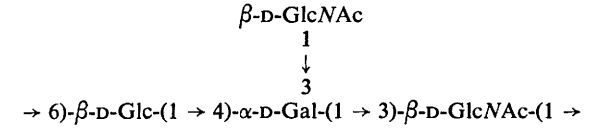
cell surface.[90] Oligosaccharides containing more than one repeating unit have been isolated from a lipopolysaccharide of *S. typhimurium*, a representative of serogroup B; the anomeric configuration of the L-rhamnosyl residues was determined by comparison of the optical rotations of the oligosaccharides with that of the trisaccharide repeating unit.[91] The results established the α-configuration of L-rhamnose units, in contrast to the β-L-configuration suggested by other workers. From a study of the rates of hydrolysis of the L-rhamnosyl linkages in a lipopolysaccharide of group D_1 *Salmonella*, it was suggested that the L-rhamnose residues have an α-configuration. The results indicated that the lipopolysaccharides of *Salmonella* groups A, B, and D_1 possess an identical main-chain polysaccharide, but differ in the configuration of 3,6-dideoxyhexose branches.

A rough strain of *S. typhimurium*, which had a defect in the inner-core region of its lipopolysaccharide, was shown to release more alkaline phosphatase into the medium during growth than did an isogenic, smooth strain of the same organism.[92] The released enzyme showed a strong tendency to associate with the purified lipopolysaccharide. Heterogeneity in the structure of a lipopolysaccharide produced by a new class of heptose-deficient mutant is assumed to be due to an altered 6-epimerase that catalyses the conversion of nucleotide-D-*glycero*-D-*manno*-heptose into nucleotide-L-*glycero*-D-*manno*-heptose in the wild form.[93] A strain of *S. typhimurium* deficient in UDP-galactose-4-epimerase produced a wild-type lipopolysaccharide only in the presence of D-galactose.[94] It was demonstrated (from double-labelling experiments) that incomplete lipopolysaccharide already in existence is not completed after the addition of D-galactose, but that only the newly synthesized lipopolysaccharide carries wild-type specificity. Using antibodies specific for the wild type, it was possible to locate the newly synthesized lipopolysaccharide over sites where the cytoplasmic and outer membranes adhere to one another. A novel glycosidase, which specifically hydrolyses polysaccharides containing α-galactosylmannosylrhamnose repeating units, was found to be associated with the *Salmonella* phage particle and might be responsible for its specific absorption into bacteria and for the degradation of polysaccharides.[95] The temperate *Salmonella* phage ε^{15} and a soluble protein present at late times in ε^{15}-infected cells were able to cleave the phage-receptor site on the surface of sensitive cells.[96] The phage receptor, a

[90] O. Stendahl, K. E. Magnusson, C. Tagesson, R. Cunningham, and L. Edebo, *Infection and Immunity*, 1973, **7**, 573.
[91] H. Kita and H. Nikaido, *J. Bacteriol.*, 1973, **113**, 672.
[92] S. S. Lindsay, B. Wheeler, K. E. Sanderson, J. W. Costerton, and K. J. Cheng, *Canad. J. Microbiol.*, 1973, **19**, 335.
[93] V. Lehmann, G. Hämmerling, M. Nurminen, I. Minner, E. Ruschmann, O. Lüderitz, T. T. Kuo, and B. A. D. Stocker, *European J. Biochem.*, 1973, **32**, 268.
[94] P. F. Muhlradt, J. Menzel, J. R. Golecki, and V. Speth, *European J. Biochem.*, 1973, **35**, 471.
[95] K. Takeda and H. Uetake, *Virology*, 1973, **52**, 148.
[96] S. Kanegasaki and A. Wright, *Virology*, 1973, **52**, 160.

heteropolysaccharide containing mannose, rhamnose, and galactose, was degraded to oligosaccharides by specific cleavage of the rhamnosyl–galactose linkages. Cross-agglutination and absorption experiments have shown that there are common antigenic determinants between *S. cholerae suis*, *S. aberdeen*, and *Metschnikowia pulcherrima*.[97] The lipid-A component of the lipopolysaccharide from *Selenomonas ruminantium* has been characterized.[98]

The O-specific side-chain polysaccharide of the endotoxin complex of *Serratia marcescens* has been shown to contain D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose in a molar ratio of 1 : 1 : 2.[99] On the basis of evidence obtained from periodate oxidation, methylation, mass spectrometry, i.r. spectroscopy, hydrazinolysis, and partial acid hydrolysis, it was concluded that the O-specific side-chain is a polysaccharide consisting of, on average, seventeen repeating units of a branched tetrasaccharide identified as (8). Heptoses have been characterized in all polysaccharide



(8)

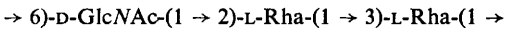
fractions obtained from strains of *Sphaerophorus necrophorus*, *S. pseudonecrophorus*, and *Eggerthella convexa*.[100] An investigation of the sugar composition of lipopolysaccharides derived from fifteen well-defined *Shigella bodyii* serotypes revealed the presence of D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, heptose, and 3-deoxyoctulosonic acid in the core polysaccharides; the O-specific side-chains contain the same sugars, with the exception of the heptose and 3-deoxyoctulosonic acid.[101] In addition to the basal sugars, D-mannose, L-rhamnose, 2-amino-2,6-dideoxy-L-glucose (L-quinovosamine), 2-amino-2-deoxy-D-galactose, and 3,6-dideoxyhexose have been found as constituents of various lipopolysaccharides. This work has resulted in a chemical classification of *S. bodyii* lipopolysaccharides into eight chemotypes differing from the accepted classification. An identical range of basal sugars is found in the lipopolysaccharides of *S. dysenteriae* and *Escherichia alcalascens-dispar* serotypes, which also contain 2-amino-2-deoxy-D-galactose, D-mannose, L-fucose, L-rhamnose, and 3,6-dideoxyhexose.[102] The sugar compositions

[97] N. Aksoycan and H. Daglioglu, *Ann. Microbiol.*, 1973, **124B**, 115.
[98] Y. Kamio, K. C. Kim, and H. Takahasi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2425.
[99] L. Tarcsay, C. S. Wang, S. C. Li, and P. Alaupovic, *Biochemistry*, 1973, **12**, 1948.
[100] B. Kedzierska, F. Meisel-Mikolajczyk, and E. Mikulaszek, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1973, **21**, 187.
[101] B. A. Dmitriev, L. V. Backinowsky, N. K. Kochetkov, and N. A. Khomenko, *European J. Biochem.*, 1973, **34**, 513.
[102] B. A. Dmitriev, L. V. Backinowsky, V. L. Lvov, N. K. Kochetkov, and I. L. Hofman, *European J. Biochem.*, 1973, **40**, 355.

of the lipopolysaccharides from serologically related *Shigella* and *Escherichia* sp. were compared, and several cross-reacting pairs were found to belong to the same chemotype. The disaccharide 2-*O*-β-D-galactopyranosyl-D-galactose has been isolated from a lipopolysaccharide obtained from *S. dysenteriae*.[103] Although the disaccharide was active in hemagglutination inhibition, a more effective inhibitor was found to be a tetrasaccharide composed of D-galactose and L-rhamnose. Both oligosaccharides are assumed to be derived from the determinant structure of the common antigen. A 3-*O*-rhamnosylrhamnose has been isolated from partial acid hydrolysates of the lipopolysaccharides from two strains of *Sh. flexneri*.[104] From this and previous investigations of the sugars present in the O-specific side-chains of these polysaccharides, the structures (9) and (10) were suggested for the repeating units. Additionally, it was shown

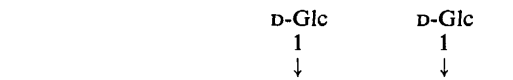


(9)



(10)

that one of the lipopolysaccharides contains large quantities of an α-(1 → 4)-linked D-glucan. Partial acid hydrolysis of the O-specific polysaccharide of *Sh. flexneri* serotype 4b-Stammes and characterization of the derived oligosaccharides indicated that the repeating unit is identical to (9), but with a single α-(1 → 4)-linked D-glucose unit on the amino-sugar.[105] The O-specific side-chain of the related *Sh. flexneri* serotype 2b-Stammes was shown to have the structure (11).[106]



(11)

The structures of *Sh. flexneri* serotypes have been reviewed.[107] Both lipopolysaccharides from *Sh. flexneri* types 3c and 4b were isolated with acetyl groups on position 2 of the L-rhamnosyl residues, and this moiety is responsible for group 6 specificity.[108] A re-investigation of the *Sh. flexneri*

[103] N. Kochibe, K. Furukawa, and S. Iseki, *Jap. J. Microbiol.*, 1973, **17**, 111.
[104] W. Beer and G. Seltmann, *Z. allgem. Mikrobiol.*, 1973, **13**, 107.
[105] G. Seltmann and W. Beer, *Z. allgem. Mikrobiol.*, 1973, **13**, 439.
[106] G. Seltmann and W. Beer, *Z. allgem. Mikrobiol.*, 1973, **13**, 349.
[107] G. Seltmann, *Z. allgem. Mikrobiol.*, 1972, **12**, 497.
[108] B. Lindberg, J. Lönngren, E. Romanowska, and U. Rudén, *Acta Chem. Scand.*, 1972, **26**, 3808.

O-antigens by methylation analysis has indicated that the side-chains of the O-specific regions of these polymers have either (12) or (13) as the

→ 3)-D-Glc*N*Ac-(1 → 2)-L-Rha-(1 → 2)-L-Rha-(1 → 3)-L-Rha-(1 →
(12)

→ 3)-D-Glc*N*Ac-(1 → 2)-L-Rha-(1 → 3)-L-Rha-(1 → 2)-L-Rha-(1 →
(13)

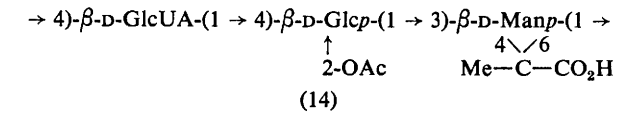
repeating unit.[109] D-Glucose linked to lipid was formed from UDP-D-glucose and ficaprenol phosphate only in strains of *Sh. flexneri* with a D-glucosylated O-antigen.[110] The polyisoprenyl-monophosphoglucose acted as a D-glucosyl donor in the formation of the specific O-antigen. Complex O-antigens have been extracted from strains of *Sh. Large-Sachs* and were characterized by their chemical, antigenic, toxic, and immunogenic properties.[111] A sugar isolated from the lipopolysaccharide of *Sh. sonnei* was characterized as a 2-amino-2-deoxyhexuronic acid.[112] Electron-microscopic examination of the lipopolysaccharides from *Treponema pallidum*, both before and after purification, has demonstrated a heterogeneous mixture of forms, one of which is a tri-laminated ribbon-form.[113] An amino-sugar constituent of the lipopolysaccharide of *Vibrio cholerae* has been identified as 2-amino-2,6-dideoxy-D-glucose (D-quinovosamine).[114] The lipopolysaccharides of the cell envelope of *V. marinus* were shown to be similar to those of R-mutants of enteric organisms with respect to the lipid-A moiety and sugars of the polysaccharide side-chain, although a high ratio of phosphate to amino-sugar was found in the lipid.[115] The lipid-A fraction of the cell-envelope lipopolysaccharide of *V. parahaemolyticus* contained 2-amino-2-deoxy-D-glucose as the only amino-sugar.[116] Evidence has been obtained for the binding of fatty acids to the hydroxy- and amino-groups of 2-amino-2-deoxy-D-glucosyl residues within the lipid-A component of the lipopolysaccharides of *V. metchnikovii* and *V. parahaemolyticus* in a similar manner to that of the endotoxins from *Salmonella* sp.[117]

A study of the adjuvant effect of a bacterial lipopolysaccharide on the IgG_1 and IgE antibodies has shown that the lipopolysaccharide induced a preferential production of the IgE antibody.[118] Lipopolysaccharides have

[109] B. Lindberg, J. Lönngren, U. Rudén, and D. A. R. Simmons, *European J. Biochem.*, 1973, **32**, 15.
[110] W. Jankowski, J. Chojnacki, and E. Janczura, *J. Bacteriol.*, 1972, **112**, 1420.
[111] M. N. Yanishevskaya and A. A. Efimova, *Zhur. Mikrobiol. Epidemiol. Immunol.*, 1973, **4**, 62.
[112] E. Romanowska and V. Reinhold, *European J. Biochem.*, 1973, **36**, 160.
[113] S. W. Jackson and P. N. Zey, *J. Bacteriol.*, 1973, **114**, 838.
[114] B. Jann, K. Jann, and G. O. Beyaert, *European J. Biochem.*, 1973, **37**, 531.
[115] C. F. Deneke and R. R. Colwell, *Canad. J. Microbiol.*, 1973, **19**, 1211.
[116] C. F. Deneke and R. R. Colwell, *Canad. J. Microbiol.*, 1973, **19**, 241.
[117] E. T. Rietschel, W. J. Palin, and D. W. Watson, *European J. Biochem.*, 1973, **37**, 116.
[118] A. Perini and I. Mota, *Immunology*, 1973, **25**, 297.

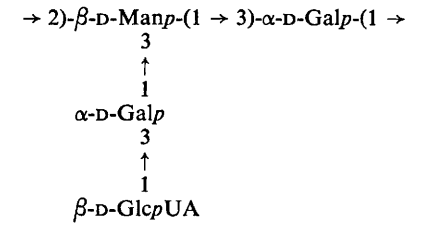
been used to stimulate immune responses to univalent haptens in spleen cultures.[119] The mitogenic effect of the lipopolysaccharide is considered to derive from its ability to complete an inductive signal to the B-lymphocytes, with two signals necessary for the induction of antibody synthesis. The lack of immunosuppressive effect on antibody production to *E. coli* lipopolysaccharides has provided evidence that anti-lympocyte serum preferentially acts on the T-lymphocytes and that enhancement occurs with some, but not all, T-helper independent antigens.[120]

Capsular Polysaccharides.—A method devised for the quantification of serum antibodies to type-specific pneumococcal polysaccharides has used the highly purified polysaccharide coated on to human O red blood cells.[121] Erythrocytes coated with acidic capsular polysaccharides of the *Klebsiella* groups have been used to determine antibodies against these type-specific polysaccharides.[122] The presence of acetate and pyruvate groups has been indicated and estimated from the ^{1}H n.m.r. spectra of *Klebsiella* capsular polysaccharides.[123] The spectra also permit an assessment to be made of the number of α- and β-linkages in the repeating unit of the polysaccharides. By use of staining techniques, polysaccharides were found to be localized inside the plasma membrane of *Diplococcus pneumoniae*, but outside the cell membrane of *K. pneumoniae*.[124] The levels of several enzymes thought to be involved in the synthesis of precursors of *K. aerogenes* types 1 and 8 polysaccharides have been examined in two wild-type strains and in non-mucoid mutants.[125] The only enzymes whose specific activities were significantly increased under conditions favouring synthesis of the polysaccharides were UDP-D-glucose pyrophosphorylase and GDP-D-mannose pyrophosphorylase. A polysaccharide (molecular weight 9×10^5) of *Klebsiella* K-type 5 was claimed to be the first *Klebsiella* polysaccharide to be isolated without a carbohydrate side-chain.[126] Methylation, periodate oxidation, and partial acid hydrolysis studies on both the native and carboxy-reduced polysaccharide revealed the presence of the repeating unit (14). A similar approach has been used to identify the repeating units (15), (16), and (17) of *Klebsiella* K-type 20,[127] K-type 21,[128] and K-type 24

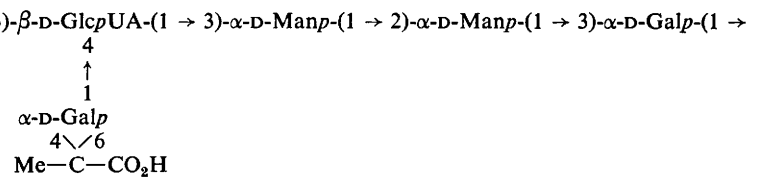


(14)

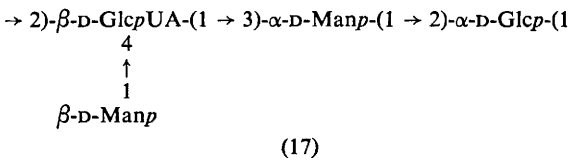
[119] J. Watson, E. Trenkner, and M. Cohn, *J. Exp. Med.*, 1973, **138**, 699.
[120] R. F. Barth, O. Singla, and P. Ahlers, *Cell Immunol.*, 1973, **7**, 380.
[121] A. J. Ammann and R. J. Pelger, *Appl. Microbiol.*, 1972, **24**, 679.
[122] J. Eriksen, *Acta Pathol. Microbiol. Scand.*, 1973, **81B**, 309.
[123] G. M. Bebault, Y. M. Choy, G. G. S. Dutton, N. Funnell, A. M. Stephen, and M. T. Yang, *J. Bacteriol.*, 1973, **113**, 1345.
[124] E. L. Springer and I. L. Roth, *J. Gen. Microbiol.*, 1973, **74**, 21.
[125] M. Norval and I. W. Sutherland, *European J. Biochem.*, 1973, **35**, 209.
[126] G. G. S. Dutton and M. Yang, *Canad. J. Chem.*, 1973, **51**, 1826.
[127] Y. M. Choy and G. G. S. Dutton, *Canad. J. Chem.*, 1973, **51**, 3015.
[128] Y. M. Choy and G. G. S. Dutton, *Canad. J. Chem.*, 1973, **51**, 198.



(15)



(16)



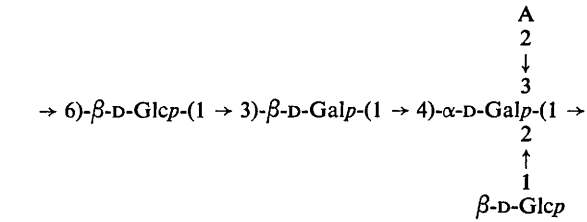
(17)

polysaccharides,[129] respectively. The configurations of the anomeric linkages were determined by ^{1}H n.m.r. spectroscopy of either isolated oligosaccharides or the carboxy-reduced and periodate-oxidized polysaccharide. Examination of the ^{1}H n.m.r. spectra also showed the presence of one *O*-acetyl group for every eight sugar residues in the K-type 20 polysaccharide, and one *O*-acetyl for every seven or eight sugar residues in the K-type 24 polysaccharide; the *O*-acetyl group was tentatively assigned to one of the D-mannosyl units in the latter case.

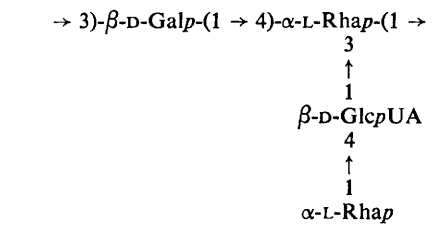
The results of methylation analysis, Smith degradation, and partial acid hydrolysis have indicated that *Klebsiella* type 38 polysaccharide is composed of a pentasaccharide repeating unit (18) containing one 3-deoxy-L-*glycero*-pentulosonic acid, two D-glucose, and two D-galactose residues, with the furanoid form of the acidic sugar linked to position 3 of a D-galactose residue.[130] The repeating units of *Klebsiella* types 47 and 56 have been identified as tetra- and penta-saccharides having structures (19) [131] and (20),[132] respectively.

Qualitative and quantitative data have been obtained on the cross-precipitation in anti-pneumococcal sera of *Klebsiella* capsular poly-

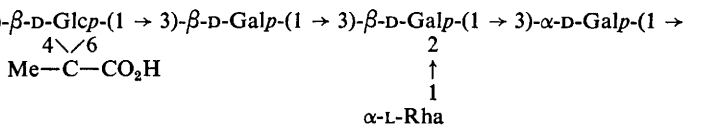
[129] Y. Choy, G. G. S. Dutton, and A. M. Zanlungo, *Canad. J. Chem.*, 1973, **51**, 1819.
[130] B. Lindberg, K. Samuelsson, and W. Nimmich, *Carbohydrate Res.*, 1973, **30**, 63.
[131] H. Björndal, B. Lindberg, J. Lönngren, K. G. Rosell, and W. Nimmich, *Carbohydrate Res.*, 1973, **27**, 373.
[132] Y. M. Choy and G. G. S. Dutton, *Canad. J. Chem.*, 1973, **51**, 3021.



(18) A = 3-Deoxy-L-*glycero*-pentulosonic acid



(19)

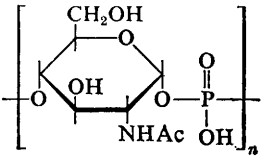


(20)

saccharides to which type numbers have been assigned.[133] Some of these reactions either confirmed structural features that had been determined already by purely chemical means or allowed predictions of possible findings. *Klebsiella* polysaccharides of known structure also permitted limited conclusions to be made regarding the nature of the linkages of sugars in capsular polysaccharides of cross-reacting pneumococcal types whose structures have not been elucidated. The development of an immune response to pneumococcal type III polysaccharide in chickens has been studied.[134] The antigen appeared to be immunogenic, inducing an antibody response that could be demonstrated by passive hemagglutination and hemolytic plaque techniques. Destruction of the D-galactose residues in pneumococcal type IV polysaccharide was approximately proportional to the extent of removal of pyruvic acid residues, with little effect on the immunological specificity until more than one-third of the D-galactose residues had been affected.[135] 2-Amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-mannose, and 2-amino-2,6-dideoxygalactose (fucosamine) residues were resistant to periodate oxidation and appeared to be separated from each other by one or more residues of (1 → 4)-linked *O*-pyruvyl-D-galactose.

[133] M. Heidelberger and W. Nimmich, *J. Immunol.*, 1972, **109**, 1337.
[134] J. Médlin, E. Feiglová, and K. Nouza, *Folia biol.*, 1973, **19**, 107.
[135] J. D. Higginbotham and M. Heidelberger, *Carbohydrate Res.*, 1973, **27**, 297.

The pyruvyl residues are acetal-linked, possibly to positions 3 and 6 of D-galactose residues. An improved procedure for the isolation of meningococcal, capsular polysaccharide antigens and also the structural determination of the antigen from serotype X have been reported.[136] The serotype X polysaccharide, containing only 2-acetamido-2-deoxy-D-glucose residues and phosphate (1 : 1), was very labile to mild acid hydrolysis, releasing 2-acetamido-2-deoxy-D-glucose 1-phosphate as the only product. Optical rotational and Fourier-transform ^{13}C n.m.r. measurements on the polysaccharide are consistent only with the α-(1 → 4)-linked polymer depicted in structure (21).



(21)

Antibody responses to meningococcal groups A and C polysaccharide vaccines have been reported.[137] Preparations of group A polysaccharide were found to be unstable on prolonged storage, and the optimal antibody response to the polysaccharides appeared to be directly related to their molecular size. Structural studies on the specific capsular polysaccharide from *Rhizobium trifolii* have indicated that the repeating unit contains one terminal residue of 4,6-*O*-(1-carboxyethylidene)-D-galactose, one residue of (1 → 3)-linked 4,6-*O*-(1-carboxyethylidene)-D-glucose, one branch point formed by a (1 → 4)-(1 → 6)-linked D-glucose, one residue of (1 → 4)-linked D-glucuronic acid, and four residues of (1 → 4)-linked D-glucose.[138] These features are accommodated in structure (22).

4,6-*O*-(1-carboxyethylidene)-D-Gal*p*
1
↓
3
4,6-*O*-(1-carboxyethylidene)-β-Glc*p*
1
↓
4
β-Glc*p*
1
↓
6
→ 4)-D-Glc*p*-(1 → 4)-D-Glc*p*-(1 → 4)-D-Glc*p*-(1 → 4)-D-Glc*p*UA-(1 → 4)-D-Glc*p*-(1 →

(22)

[136] D. R. Bundle, H. J. Jennings, and C. P. Kenny, *Carbohydrate Res.*, 1973, **26**, 268.
[137] B. L. Brandt, M. S. Artenstein, and C. D. Smith, *Infection and Immunity*, 1973, **8**, 590.
[138] A. S. Chaudhari, C. T. Bishop, and W. F. Dudman, *Carbohydrate Res.*, 1973, **28**, 221.

Exopolysaccharides of *R. meliloti* and *Agrobacterium tumefaciens*, containing D-glucose, D-galactose, pyruvic acid, and *O*-acetyl groups (6 : 1 : 1 : 1.5), have been analysed by methylation. They were found to contain (1 → 3)-linked D-galactose and (1 → 3)-, (1 → 4)-, and (1 → 6)-linked D-glucose residues, with branching on some of the D-glucose residues at positions 4 and 6.[139] The terminal D-glucose residues were shown to be substituted at positions 4 and 6 by pyruvate. D-Glucuronic acid residues were reported to be (1 → 4)-linked to other hexoses in the polysaccharides of *R. leguminosarum*, *R. phaseoli*, and *R. trifolii*. When the acidic capsular polysaccharide of *Vibrio parahaemolyticus* K15 antigen was esterified and reduced, the hydrolysates contained two amino-sugars that were identified as 2-amino-2-deoxymannose and 2-amino-2-deoxygulose on the basis of paper and ion-exchange chromatography.[140] This implied that the K15 antigen contains 2-amino-2-deoxymannuronic acid and 2-amino-2-deoxyguluronic acid; confirmation was obtained by liberation of the two aminouronic acids on direct hydrolysis of the antigen.

Extracellular and Intracellular Polysaccharides.—The compositions of cellular and extracellular polysaccharides of bacterial Gram-positive and Gram-negative organisms have been reviewed.[141] A polysaccharide containing glucose, galactose, mannose, and probably glucuronic acid was elaborated by an *Achromobacter* sp.[142] An extracellular polysaccharide (molecular weight 3.5×10^4) composed of 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and D-glucose (5 : 3 : 1) was formed by *Bacillus cereus*.[143] Characterization of a mucoid strain of *Escherichia coli* K12 by the over-production of an extracellular polysaccharide, containing fucose, glucose, galactose, and possibly glucuronic acid, has been reported.[144] Improved yields of colominic acid, a homopolymer of *N*-acetylneuraminic acid, have been obtained by growing *E. coli* on D-glucitol as a source of carbon. The purified polysaccharide was found to be devoid of internal ester linkages.[145] Protein-free, extracellular, acidic heteroglycans have been synthesized by *Mycobacterium lacticolum*.[146] Carbohydrate antigens from a number of non-pathogenic *Neisseria* sp. have been examined by means of chromatography, membrane filtration, and enzymic degradation.[147] The antigens were shown to be polymers of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose, the latter only being a constituent

[139] L. P. T. M. Zevenhuizen, *Carbohydrate Res.*, 1973, **26**, 409.
[140] M. Torii, K. Sakakibara, and K. Kuroda, *European J. Biochem.*, 1973, **37**, 401.
[141] B. Lindberg and S. Svensson in 'Carbohydrates', ed. G. O. Aspinall, in 'MTP International Review of Science, Series One', Butterworths, 1973, Vol. 7.
[142] Y. Ozawa, K. Yamada, K. Kobayashi, and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2117.
[143] D. Mirleman, R. Lotan, Y. Bernstein, H. M. Flowers, and N. Sharon, *J. Gen. Microbiol.*, 1973, **77**, 5.
[144] S. Shugar and K. E. Sanderson, *Canad. J. Microbiol.*, 1972, **18**, 969.
[145] Y. Uchida, Y. Tsukada, and T. Sugimori, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2105.
[146] E. V. Gogoleva, N. N. Grechushkina, and N. S. Egorov, *Mikrobiol.*, 1973, **42**, 409.
[147] G. H. Wagner, F. P. Cooper, and C. T. Bishop, *Canad. J. Microbiol.*, 1973, **19**, 703.

of the antigens from *N. caviae* and *N. sicca*. A glucuronomannan from *Lypomyces lipofer* was shown to have the structure (23) on the basis of methanolysis, Hoffman degradation of the carboxyamide of the methylated polysaccharide, and oxidation of the carboxy-reduced polysaccharide acetate.[148]

$$\rightarrow 4)\text{-D-Glc}p\text{UA-}(1 \rightarrow 3)\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-D-Man}p\text{-}(1 \rightarrow$$
(23)

The staining of a marine bacterium with Ruthenium Red or Alcian Blue has demonstrated the existence of an extracellular, compact, acidic polysaccharide layer that is involved in the adhesion of the bacterial cells to surfaces.[149]

The thermodynamic incompatability of gelatin with D-glucans such as dextran in isoionic conditions is determined by self-association of the gelatin macro-ions owing to interaction of charge fluctuations.[150] The thermal diffusion of dextrans has been studied by experimental and computational procedures designed to minimize the effects of convection.[151] Contrary to the conclusions reached from thermogravitational studies, no transport by thermal diffusion was observed for dextran in distilled water and salt solutions, even though such transport has been observed for polystyrene in toluene with similar temperature gradients. Calcium ions have been reported to bind with low affinity to dextrans, but in amounts sufficient to influence markedly the rate of dialysis of these ions.[152] Dextrans (molecular weight 1×10^5) have been studied for polydispersity by t.l.c. after combination with triazine dyes.[153] ^{1}H N.m.r. spectroscopy of dextrans and their derivatives has been reported.[154] Methanolysates of permethylated dextran have been oxidized to give the corresponding dicarbonyl sugars, which were then converted into the corresponding 2,4-dinitrophenylhydrazones.[155] High-resolution mass spectrometry of the separated hydrazones showed distinctive characteristics. A Smith-degradation procedure, modified by the introduction of a methylation step after borohydride reduction, has been applied to studies of two clinical dextrans.[156] Dextrans from *Leuconostoc mesenteroides* reacted with the antisera to ribitol and glycerol teichoic acids from *Lactobacillus plantarum*; the antibodies are specific for α-D-glucose substituents of the teichoic acids.[17] Dextranase has been used to remove the interference caused by

[148] N. K. Kochetkov, S. E. Gorin, A. F. Sviridov, O. S. Chizov, V. L. Golubev, I. P. Babeva, and A. Y. Podelko, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2304.
[149] M. Fletcher and G. D. Floodgate, *J. Gen. Microbiol.*, 1973, **74**, 325.
[150] V. Y. Grinberg and V. B. Tolstoguzov, *Carbohydrate Res.*, 1972, **25**, 313.
[151] F. J. Bonner, *Chem. Scripta*, 1973, **3**, 149.
[152] K. C. Reed, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 1136.
[153] P. T. Aspinall and J. N. Miller, *Analyt. Biochem.*, 1973, **53**, 509.
[154] B. Coxon, *Adv. Carbohydrate Chem. Biochem.*, 1972, **27**, 39.
[155] N. Kashimura, K. Yoshida, and K. Onodera, *Carbohydrate Res.*, 1972, **25**, 264.
[156] P. Nánási and A. Lipták, *Carbohydrate Res.*, 1973, **29**, 193.

dextran in the biuret determination of serum proteins.[157] Three trisaccharides (24)—(26) have been isolated and characterized following acetolysis of a dextran.[158]

α-D-Glc*p*-(1 → 2)-α-D-Glc*p*-(1 → 6)-D-Glc
(24)

α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 2)-D-Glc
(25)

α-D-Glc*p*-(1 → 2)-[α-D-Glc*p*-(1 → 6)]-D-Glc
(26)

Dextrans have been detected unambiguously in mixtures with starch and other D-glucans by the formation of isomaltose on digestion with a fungal dextranase.[159] A series of end-labelled isomaltose oligosaccharides has been prepared by the reaction of dextran-sucrase with [^{14}C]sucrose in the presence of an excess of unlabelled isomaltose saccharides as alternative acceptors.[160] The end-labelled saccharides were used to determine the specificity of a bacterial dextranase that required five or more consecutive α-(1 → 6)-D-glucosidic linkages in the substrate. A soil bacterium has been found to degrade dextrans to D-glucose principally, but was unable to attack certain non-(1 → 6)-linkages.[161] Native dextrans with α-(1 → 6)- and α-(1 → 3)-bonds were degraded by purified acid α-glucosidase from pig spleen, although considerable difficulty was found in obtaining cleavage of α-(1 → 2)-linkages.[162] From the rates of hydrolysis of the substrates, suggestions were made as to possible differences in structure of a number of dextrans. A crude dextranase from *Penicillium funiculosum* was found to inhibit the formation of insoluble glucan by *Streptococcus mutans*.[163] Dextrans were incompletely degraded by an α-1,6-glucan glucanohydrolase from *S. mitis*, and the extent of hydrolysis is related to the proportion of α-(1 → 6)-D-glucosidic linkages.[164] It was concluded that the α-(1 → 4)-, α-(1 → 3)-, and α-(1 → 2)-D-glucosidic linkages that occur in bacterial dextrans are resistant to the action of the dextran-glucosidase, and arrest any further degradation of the dextran. Antidextrans of α-(1 → 2)-specificity from humans and rabbits have been further characterized, with regard to their combining sites, by means of inhibition assays with the trisaccharides (24)—(26).[165] After absorption of the α-(1 → 6)-specific

[157] S. M. Nazar and H. Schmidt, *Z. klin. Chem. klin. Biochem.*, 1972, **10**, 548.
[158] K. Sakakibara, M. Torii, A. Misaki, and H. Miyaji, *Carbohydrate Res.*, 1972, **25**, 443.
[159] W. Gold, F. B. Preston, and H. Blechman, *J. Pediodontol.*, 1973, **44**, 263.
[160] G. J. Walker, *Carbohydrate Res.*, 1973, **30**, 1.
[161] N. W. H. Cheetham and G. N. Richards, *Carbohydrate Res.*, 1972, **25**, 333.
[162] M. E. Preobrazhenskaya and E. L. Rosenfeld, *Biokhimya*, 1972, **37**, 974.
[163] N. Kosaric, K. Yu, J. E. Zajic, and J. Rozanis, *Biotechnol. Bioeng.*, 1973, **15**, 729.
[164] G. J. Walker and A. Pulkownik, *Carbohydrate Res.*, 1973, **29**, 1.
[165] M. Torii, K. Sakakibara, and E. A. Kabat, *J. Immunol.*, 1973, **110**, 951.

antibody, human-antidextran sera contained antibodies complementary to trisaccharides (24), whereas rabbit antisera contained antibodies complementary to both trisaccharides (24) and (25). The trisaccharides were not the best inhibitors of human antidextrans having non-α-(1 → 2) specificity. The cross-reaction of horse antipneumococcal type XII antiserum was best inhibited by the trisaccharide (24). Kojibiose α-octa-acetate has been isolated from a dextran and converted into *p*-nitrophenyl and *p*-isothiocyanatophenyl β-kojibioside.[166] The effect of dextrans of different mean molecular weights on the immunodiffusion of serum fractions has been reported.[167]

Cyclomalto-hexaose and -heptaose have been reported to be competitive inhibitors of pullulanase.[168] Decarboxylation rate constants in aqueous solutions containing cycloamyloses have been determined for a number of variously substituted phenylcyanoacetate anions.[169] All data were consistent with catalysis by cyclohepta-amylose arising from the solvation change experienced by the carboxylate anion on transfer from an aqueous environment to the cyclohepta-amylose complex. Decarboxylation of benzoylacetic acids was accelerated in the presence of cyclohepta-amylose, and the results were again interpreted in terms of a microsolvent effect arising from substrate–cycloamylose binding by inclusion of the substrate in the cycloamylose cavity.[170]

Glycogen produced by thermophilic bacteria[171] and the regulatory mechanisms of biosynthesis of bacterial glycogen have been reviewed.[172] An intracellular α-D-glucan from *Clostridium botulinum* is considered to have a ramified structure, composed of chains of α-(1 → 4)-D-gluco-pyranosyl residues with α-(1 → 6)-linkages at branch points, in a molecule resembling amylopectin rather than glycogen.[173] A particulate fraction of an *E. coli* mutant has been found to catalyse the transfer of D-glucose from ADP-D-glucose not only to glycogen, but also to a methanol-insoluble product in the absence of primer.[174] The latter reaction required the presence of albumin and either high concentrations of salts or a protein factor. Increased rate and extent of glycogen synthesis were found to occur in a mutant, compared with the parent strain, of *E. coli* owing to an alteration in the regulatory properties of the ADP-glucose synthetase.[175] It appears that the physiologically important activator for the synthetase is D-fructose

[166] J. Duke, N. Little, and I. J. Goldstein, *Carbohydrate Res.*, 1973, **27**, 193.
[167] N. St. G. Hyslop, *J. Chromatog.*, 1973, **77**, 445.
[168] J. J. Marshall, *F.E.B.S. Letters*, 1973, **37**, 269.
[169] T. S. Straub and M. L. Bender, *J. Amer. Chem. Soc.*, 1972, **94**, 8875.
[170] T. S. Straub and M. L. Bender, *J. Amer. Chem. Soc.*, 1972, **94**, 8881.
[171] S. H. Goldemberg in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[172] J. Preiss in 'Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[173] J. N. C. White and G. A. Strasdine, *Carbohydrate Res.*, 1972, **25**, 435.
[174] J. P. Chambost, A. Favard, and J. Cattanéo, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 132.
[175] S. Govons, N. Gentner, E. Greenberg, and J. Preiss, *J. Biol. Chem.*, 1973, **248**, 1731.

1,6-diphosphate. The intracellular storage polysaccharide of the rumen organism Eadie's Oval has been purified and found to be a D-glucan of the glycogen type containing solely α-(1 → 4)- and α-(1 → 6)-linked D-glucose units.[176] It was found to be highly branched, with mean exterior and interior chain lengths of 7 and 3, respectively, and polydisperse, with a mean molecular weight of 4.3×10^6. A β-(1 → 3)-glucan produced from culture filtrates of *Alcaligenes faecalis* possesses the unique property of forming a gel on heating.[177] Heating of aqueous suspensions of the polysaccharide produced an elastic gel that was stable to heat and to acid. Degradation of the gel-forming glucan produced β-(1 → 3)-linked glucans of various degrees of polymerization.[178] O.r.d. studies indicated that polymers of $DP_n < 25$ assumed a disordered form in both neutral and alkaline solutions, whereas those of higher DP_n took on an ordered structure in dilute alkali, with the proportion of ordered structure increasing with DP_n to attain a maximum value at a DP_n of around 200. The formation of complexes with Congo Red in alkaline solutions supported these conclusions. A conformational transition of this glucan from a flexible, disordered to a rigid, ordered form was observed with increasing mole fractions of 2-chloroethanol, p-dioxan, and water.[179]

Miscellaneous Bacterial Polysaccharides.—A membrane-bound polysaccharide containing 2-amino-2-deoxy-D-glucose has been extracted from *Acholeplasma laidlawii* with aqueous ethanol.[180] However, the polysaccharide does not appear to be covalently attached to membrane proteins, but is weakly associated with the membrane and can reside on or near the exterior surface. A phosphorylated polysaccharide, containing 2-acetamido-2-deoxy-D-galactose, D-glucose, and phosphate, on controlled hydrolysis released 2-acetamido-2-deoxy-3-*O*-β-D-glucopyranosyl-D-galactose 6′-phosphate, with the phosphomonoester group at position 6 of the D-glucose residue.[181] The polysaccharide is considered to consist of disaccharide units, with phosphodiester residues joining position 1 of the amino-sugar to position 6 of the D-glucose residue in a neighbouring unit. A polysaccharide extracted from the cell walls of *Bacillus licheniformis* has been shown previously to contain an unusual diamino-sugar named *N*-acetylbacillosamine (see N. Sharon and R. W. Jeanloz, *J. Biol. Chem.*, 1960, **235**, 1). The configurations of this sugar at C-4 and C-5 were determined by its oxidation to D-allothreonine, and that at C-2 by its conversion into *N*-(2,4-dinitrophenyl)-L-serine. These results and those of ^1H n.m.r. spectroscopy of the fully acetylated derivative identified *N*-acetylbacillosamine as 4-acetamido-2-amino-2,4,6-trideoxy-D-glucose.[182]

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[179] K. Ogawa, M. Miyagi, T. Fukumoto, and T. Watanabe, *Chem. Letters*, 1973, 943.
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[182] U. Zehavi and N. Sharon, *J. Biol. Chem.*, 1973, **248**, 433.

Spores of *Clostridium botulinum* contain a carbohydrate hapten associated with the spore coat that is connected with the maintenance of rigidity.[183] The cell walls of *Mycobacterium lepraemurium* contain five subunits from which arabinose, mycolic acids, a tetrapeptide (Ala-Gln-DAP-Ala), a disaccharide [4-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramic acid], and an arabinogalactan have been obtained.[184] The cell wall is considered to be composed of a mycolic acid–arabinogalactan–mucopeptide complex. Complex formation between the polysaccharide from *M. phlei*, which contains numerous *O*-methyl sugar residues, and the coenzyme A derivatives of C_{18}, C_{20}, and C_{22} fatty acids yielded complexes containing maximally one mole of acylated coenzyme A per mole of polysaccharide.[185] The formation of these novel complexes is considered to result from hydrophobic interactions between the hydrocarbon chains of the acylated coenzyme A derivatives and the *O*-methyl sugar residues of the polysaccharide. An arabinose phosphate isolated from the walls of *M. tuberculosis* has been tentatively identified as D-arabinofuranose 1-phosphate.[186] Partial acid hydrolysis of a cell-wall arabinogalactan isolated from *M. tuberculosis* released a disaccharide that was identified as 6-*O*-β-D-galactofuranosyl-D-galactose on the basis of periodate oxidation, ^{1}H n.m.r. spectroscopy, and g.l.c.–mass spectrometry of the permethylated derivative.[187] The compositions of somatic and flagellar components of *Pasteurella pseudotuberculosis* have been reported.[188] The purified polysaccharides from five serological groups were all shown to contain D-glucose, D-galactose, D-xylose, and 2-amino-2-deoxy-D-glucose, with three other sugars distributed between the groups.

The cell-wall antigen from *Streptococcus mutans* has been extracted from cell walls and whole cells, and was found to contain L-rhamnose, D-galactose, and 2-amino-2-deoxy-D-glucose in the ratio of 5 : 2 : 1.[189] The polysaccharide antigen isolated from *S. mutans* has a molecular weight of 1×10^5 and contains the *S. mutans* group 'a' antigenic site as well as a second antigenic site that is common to 'a' and some group 'd' strains.[190] Electrophoretic, chromatographic, and immunological data indicated that the two sites exist in a single molecule composed of D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose. Immunological specificity to the group 'a' polysaccharide was shown to depend primarily on a D-glucosyl-D-glucose sequence, and to the 'a–d'

[183] R. Z. Hawirko, K. L. Chung, A. J. C. Magnusson, and A. C. Emeruwa, *J. Bacteriol.*, 1972, **112**, 1416.
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[185] Y. Machida and K. Bloch, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 1146.
[186] C. Amar and E. Vilkas, *Compt. rend.*, 1973, **277**, *D*, 1949.
[187] E. Vilkas, C. Amar, J. Markovitz, J. F. G. Vliegenthart, and J. P. Kamerling, *Biochim. Biophys. Acta*, 1973, **297**, 423.
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[189] T. E. Burgess and J. R. Edwards, *Infection and Immunity*, 1973, **8**, 491.
[190] H. Mukasa and H. D. Slade, *Infection and Immunity*, 1973, **8**, 190.

site on a terminal D-galactose sequence. The antigen from *S. mutans* group 'b' contains a polysaccharide and a glycoprotein that could be separated by gel immunoelectrophoresis.[191] The polysaccharide was shown to contain L-rhamnose, D-galactose, and 2-amino-2-deoxy-D-galactose, with the latter two sugars being strong inhibitors of the precipitation reaction. Agglutination of whole cells by specific group 'b' antiserum indicated that the antibody-receptor sites of the polysaccharide antigen are at the surface of the streptococcal cell. Transfers of *S. pyogenes* treated with mitomycin C produced a group A streptococcal variant that reacted with group C antiserum; the variant contained 2-amino-2-deoxy-D-galactose, but not 2-amino-2-deoxy-D-glucose, whereas the parent strain shows the reverse pattern.[192] A number of other variants contained both amino-sugars and D-glucose. The strains containing 2-amino-2-deoxy-D-galactose possessed UDP-2-acetamido-2-deoxy-D-glucose 4-epimerase activity, which is absent in the parent strain. The active sites of rabbit-derived antibodies to group A streptococcal polysaccharide have been affinity-labelled with *p*-diazoniumphenyl 2-acetamido-2-deoxy-β-D-glucoside.[193] A random and related group of rabbits has been used for the production of antibodies to streptococcal group A-variant polysaccharide.[194] The results suggested that the immune response to the A-variant polysaccharide is controlled by genetic factors that also influence the degree of heterogeneity. A streptococcal strain, classified as Z_3 III, was differentiated from its mutant strain Z_3, lacking the type III polysaccharide antigen, by Curie-point g.l.c.[195] Differences were observed in the pyrograms of either whole cells or cell envelopes of both strains that could be directly related to the pattern of pyrolysis of the purified type III antigen. Extracted polysaccharides of Group F streptococci were separated into the group Z_3 antigen, containing L-rhamnose (50%) and amino-sugars, and the type III antigen, containing L-rhamnose, D-glucose, and D-galactose (1 : 2 : 3).[196] From the characterization of oligosaccharides released from the type III antigen, the most probable structure (27) of the determinant group was deduced.

β-D-Glc-(1 → 6)-D-gal-(1 → 3)-L-Rha

(27)

Electron-microscopic studies of streptococci stained with ferritin-labelled antisera showed that the type antigens are located on the outermost layer of the cell envelope and seem to be capsule-like materials, and

[191] H. Mukasa and H. D. Slade, *Infection and Immunity*, 1973, **7**, 578.
[192] T. Hirano, T. Itoh, T. Tomura, and M. Yoshioka, *Jap. J. Microbiol.*, 1973, **17**, 53.
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[194] D. G. Braun, E. Kjems, and M. Cramer, *J. Exp. Med.*, 1973, **138**, 645.
[195] J. H. J. Huis In'T Veld, H. L. C. Meuzelaar, and A. Tom, *Appl. Microbiol.*, 1973, **26**, 92.
[196] J. M. N. Willers, M. F. Michael, J. H. J. Huis In'T Veld, and G. H. J. Aldercamp, *J. Microbiol. Serol.*, 1973, **39**, 369.

that the group antigens are located more to the inside of the streptococcal envelope.[197] Immunochemical studies performed on group-specific carbohydrates produced by streptococcal variants at different temperatures have revealed that the appearance of group-A serological activity at lower temperatures is due to the synthesis of an additional polysaccharide with group-A specificity, together with the continued synthesis of the variant carbohydrate.[198] This finding contrasted with data obtained on the carbohydrate produced by intermediate organisms that appears to consist predominantly of one molecule bearing dual A and variant determinants.

Fungal Cell Walls

The strategy and tactics involved in the preparation and quantitative analysis of fungal cell walls have been reviewed.[199] A wide diversity of treatments has been shown to cause rapid and extensive bursting of hyphal tips, with the possible involvement of biochemical reactions as rate-limiting steps.[200] It was concluded that the growing tips of fungi have a large wall-lytic potential, the release of which during growth must be gradual and delicately co-ordinated with wall synthesis. This balance can be easily disturbed by a wide variety of external stimuli, with the ensuing surge of lytic activity resulting in the violent disintegration of the hyphal apex. The extent of digestion of walls of Basidomycetes by an enzyme preparation of *Streptomyces* and a preparation containing glucanase and chitinase was found to vary appreciably.[201] Evidence suggested that enzymes additional to glucanase and chitinase are needed for lysis of these fungi, and that resistance to lysis could be associated with the presence of wall polysaccharides containing monomers in addition to D-glucose and 2-acetamido-2-deoxy-D-glucose. Hyphae of *Aspergillus nidulans* continued to synthesize all major polysaccharide components of the cell wall, *viz.* an α-(1 $\rightarrow$ 3)-glucan, a β-(1 $\rightarrow$ 3)-glucan, and chitin, after cyclohexamide had been added to the cultures.[202] Electron-microscopic examination showed that the hyphae did not elongate, but that the walls became thicker around the cell; it was concluded that cycloheximide changes the synthesis of walls from extension at the apex to subapical thickening. A possible method for estimating the rate of accumulation of walls by regenerating protoplasts has been proposed on the basis of a linear relationship observed between the adsorption of fluorescent brighteners and the amount of wall present.[203] The yeast-like walls of *Aureobasidium pullulans* have been shown to contain D-glucose, D-galactose, and D-mannose, whereas the major component of the chlamydospore walls is D-glucose, occurring in linear chains of

[197] J. H. J. Huis In'T Veld and W. H. Linssen, *J. Gen. Microbiol.*, 1973, **74**, 315.
[198] E. M. Ayoub and B. A. Dudding, *J. Exp. Med.*, 1973, **138**, 117.
[199] I. E. P. Taylor and D. S. Cameron, *Ann. Rev. Microbiol.*, 1973, **27**, 243.
[200] S. Bartnicki-Garcia and E. Lippman, *J. Gen. Microbiol.*, 1973, **73**, 487.
[201] J. P. G. Ballesta and M. Alexander, *Trans. Brit. Mycol. Soc.*, 1972, **58**, 481.
[202] E. Sternlicht, D. Katz, and R. F. Rosenberger, *J. Bacteriol.*, 1973, **114**, 819.
[203] J. F. Peberdy and C. E. Buckley, *J. Gen. Microbiol.*, 1973, **74**, 281.

(1 → 3)- and (1 → 6)-linked units.[204] A number of plant agglutinins were found to agglutinate the blastospores of a pathogenic strain of *Candida albicans*.[205] Concanavalin A also caused agglutination, through α-D-glucosyl and α-D-mannosyl linkages, as did certain anti-H factors of plant origin. These results suggest that the yeast must bear receptors having chemical structures similar to that of blood-group H substance.

Mycelial and blastophore forms of *C. albicans* have been grown under conditions in which only the temperature was varied.[206] A requirement of NADPH for cell division was indicated by measurements of the phosphoglucose isomerase and phosphofructokinase activities, and from the metabolism of D-[^{14}C]glucose. It was suggested that control over the syntheses of chitin and mannan may be provided through control of the phosphofructokinase activity by adenosine phosphates.

Knowledge of the structure of the cell walls of *Candida* sp. has been demonstrated to have possible taxonomic value. It was observed that the main group of thirty-seven species of *Candida* have a rigid, cell-wall structure, consisting of a network of β-glucans covered by a layer of proteins rich in disulphide bonds, are able to grow on D-glucose, and have no urease activity.[207] A second group were not hydrolysed by β-(1 → 3)-glucanase and did not grow on D-glucose, but contained urease activity. Methylation analysis has shown that each of the rhamnomannans from *Ceratocystis stenoceras* and *Sporothrix schenckii* possesses a gross structure similar to that of *C. ulmi* in having a main chain of (1 → 6)-linked α-D-mannopyranosyl residues substituted at O-3 by α-L-rhamnopyranosyl and, in many cases, by α-L-rhamnopyranosyl-(1 → 2)-L-rhamnopyranosyl side-chains.[208] Differences among polysaccharides of the *Ceratocystis* sp. were indicated to reside in the relative numbers of dirhamnosyl side-chains and of 4-*O*- and 2,4-di-*O*-substituted D-mannose units. Compositional studies of the cell walls of the synnema and vegetative hyphae of *C. ulmi* showed a higher concentration of carbohydrate material in the synnemal wall; sugars identified were D-glucose, D-mannose, D-galactose, and L-rhamnose.[209] Enzymic hydrolysis of both types of wall by cellulase and laminarinase indicated the presence of β-(1 → 3)- and β-(1 → 4)-linked polymers of D-glucose; 2-acetamido-2-deoxy-D-glucose was liberated by chitinase.

The chemical composition and submicroscopic morphology of the hyphal cell walls of the Ascomycete *Chaetomium globosum* have been studied.[210] The walls are composed of polysaccharides, including chitin and

[204] R. G. Brown, L. A. Hanic, and M. Hsiao, *Canad. J. Microbiol.*, 1973, **19**, 163.
[205] W. P. Herrmann and G. Uhlenbruck, *Z. Naturforsch.*, 1972, **27b**, 1284.
[206] F. W. Chattaway, R. Bishop, M. R. Holmes, F. C. Odds, and A. J. E. Barlow, *J. Gen. Microbiol.*, 1973, **75**, 97.
[207] M. Bastide, P. Trave, and J. M. Bastide, *Ann. Microbiol.*, 1973, **124A**, 359.
[208] L. R. Travassos, P. A. J. Gorin, and K. O. Lloyd, *Infection and Immunity*, 1973, **8**, 685.
[209] J. L. Harris and W. A. Taber, *Canad. J. Bot.*, 1973, **51**, 1147.
[210] R. Maret, *Arch. Mikrobiol.*, 1972, **81**, 68.

a glucan with both β-(1 → 3)- and β-(1 → 6)-linkages, protein, and lipid. Serine, threonine, and aspartic acid components of the protein are thought to be involved in protein–polysaccharide linkages. A periodate–thiosemicarbazide–silver proteinate method and metal-shadowing techniques have been used in an attempt to localize the individual polymeric constituents of the cell wall of *Dendryphiella salina* in its native state and after various alkaline extractions and enzymic digestions, when 3-*O*-methyl-D-glucose was actively transported into the cells. Metabolizable, soluble carbohydrate was found to be converted into polysaccharide and other insoluble material in such a way that the total, soluble carbohydrate remained constant.[211] The outermost of the four spore wall-layers of *Dictyostelium discoideum* was shown to be amorphous by electron microscopy.[212] D-Galactose was found to be the major component of the acidic polysaccharide in this layer. Cell walls of a strain of *Micromonospora* contain a polysaccharide composed of D-xylose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and phosphate.[213] The results of structural studies suggested the existence of a branched polysaccharide, mainly containing (1 → 3)-linkages and with phosphoric acid esterifying position 6 of the amino-sugar. Macroconidial walls of *Neurospora crassa* have been shown to contain substantial proportions of a polysaccharide containing 2-amino-2-deoxy-D-galactose, compared with a significant reduction in the contents of β-glucan and chitin of the conidia.[214] Comparative ultrastructural analysis of the walls, including selective digestion, revealed three layers of unequal thickness, *viz.* an internal, alkali-soluble layer presumed to be glycoprotein, a thin, median layer, possibly containing chitin, and an external layer. In the latter layer, alkali- and acid-resistant β-glucans and chitin microfibrils were found to be loosely packed into an aveolar network embedded in a thick, alkali-soluble matrix of glycoprotein. An acidic polysaccharide, containing 2-amino-2-deoxy-D-galactose, D-glucose, and protein (100 : 8 : 8), has been isolated from a *Neurospora* sp. and was shown to possess considerable antitumour activity.[215] Conidial and mycelial cell walls of *Penicillium notatum* were found to differ mainly in the distribution of amino-acids, neutral sugars, and amino-sugars.[216] D-Glucose, D-galactose, D-mannose, and L-rhamnose were detected in the walls of resting spores, but L-rhamnose was absent at other stages of germination. To study the changes in the cell walls of this species during germination, thin sections of resting, swollen, and germinating spores and mycelium were compared with sections of the isolated cell walls.[217] Degradation of

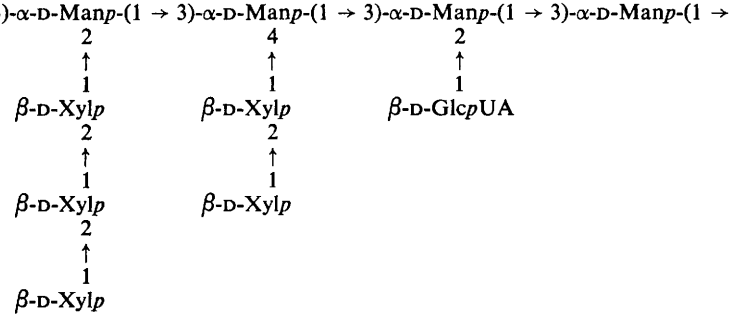
[211] D. H. Jennings and S. Austin, *J. Gen. Microbiol.*, 1973, **75**, 287.
[212] D. E. Hemmes, E. S. Kojima-Buddenhagen, and H. R. Hohl, *J. Ultrastruct. Res.*, 1972, **41**, 406.
[213] H. Tabaud, J. C. Massot, and E. Vilkas, *Biochemie*, 1973, **55**, 605.
[214] N. Coniordos and G. Turian, *Ann. Microbiol.*, 1973, **124A**, 5.
[215] J. L. Klein, J. E. Glasgow, N. Meiselman, and J. L. Reissig, *J. Cell Biol.*, 1972, **52**, 139A.
[216] J. F. Martin, G. Nicolas, and J. R. Villanueva, *Canad. J. Microbiol.*, 1973, **19**, 789.
[217] J. F. Martin, F. Uruburu, and J. R. Villanueva, *Canad. J. Microbiol.*, 1973, **19**, 797.

Phytophthora cactorum mycelial mats has been effected by incubation with a mixture of carbohydrases, and resulted in the production of oospore suspensions free from mycelial debris.[218] Apical walls in rapidly growing cultures of *P. parasitica* were shown to be significantly thinner than mature, non-apical walls.[219] Enzymic treatment has shown that, in young species, the outer layer of wall glucan is attenuated compared with mature regions, and it was suggested that there is less protein associated with the cellulose microfibrils in the inner part of the wall. The content of 2-amino-2-deoxy-D-glucose in the walls of *Saccharomyces cerevisiae* cells without bud scars and with multiple scars has been examined.[220] Results of i.r. and *X*-ray analyses of the cell-wall fractions have indicated the presence of α-chitin as part of a glucan–chitin complex. Electron microscopy demonstrated that the complex has a fibrillar arrangement and that it is localized in the encircling region of the bud scar.[221] There are indications of the presence of chitin in the primary septum. The release of protoplasts from young mycelia of *Schizophyllum commune* by a lytic enzyme preparation from *Trichoderma viride* was accompanied by the degradation of β-glucan, α-(1 → 3)-glucan, and chitin.[222] Part of the α-(1 → 3)-glucan was resistant to enzymic attack; the resistance increased with the age of the culture and was accompanied by a concomitant reduction in the yield of protoplasts. It was concluded that the α-(1 → 3)-glucan protects chitin and, possibly, the β-glucan in the living cell against degradation by external enzymes. Analysis of isolated cell walls of *Schizosaccharomyces pombe* grown in the presence of 2-deoxy-D-*arabino*-hexose showed marked changes in composition, mainly through a decreased content of both D-glucose and 2-amino-2-deoxy-D-glucose, compared with untreated cells.[223] A related study has been made of changes in the cell walls and in the ultrastructure of cells and regenerating protoplasts of *S. pombe* grown in varying concentrations of 2-deoxy-D-*arabino*-hexose.[224] The cell wall of *Smittium culisetae* has been found to contain 65% polysaccharide, primarily composed of D-glucose, 2-amino-2-deoxy-D-glucose, and D-galactose.[225] Evidence was obtained for the presence of chitin.

When *Polyporus circinatus* was grown on D-glucose as a source of carbon, an intracellular polysaccharide, containing D-mannose, D-glucose, D-galactose, and D-glucuronic acid, was produced.[226] The corresponding expopolysaccharide is composed of the same sugars, with the exception of D-glucuronic acid. An acidic polysaccharide isolated from culture media of *Tremella mesenterica* was shown to contain D-xylose, D-mannose,

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[219] D. Hunsley, *New Phytologist*, 1973, **72**, 985.
[220] K. Beran, Z. Holan, and J. Baldrian, *Folia Microbiol.*, 1972, **17**, 322.
[221] O. Seichertová, K. Beran, Z. Holan, and V. Pokorny, *Folia Microbiol.*, 1973, **18**, 207.
[222] O. H. M. De Vries and J. G. H. Wessels, *J. Gen. Microbiol.*, 1973, **76**, 319.
[223] R. K. Poole and D. Lloyd, *Arch. Mikrobiol.*, 1973, **88**, 257.
[224] A. Svoboda and D. G. Smith, *Z. allgem. Mikrobiol.*, 1972, **12**, 685.
[225] V. K. Sangar and P. R. Dugan, *Mycologia*, 1973, **65**, 421.
[226] M. H. Pinotti and G. T. Zancan, *Rev. Microbiol.*, 1972, **3**, 35.

D-glucuronic acid, and *O*-acetyl residues (7 : 5 : 1 : 0.7).[227] Methylation analysis and other data indicated the presence of an α-(1 → 3)-linked D-mannopyranose backbone, with approximately 80% of these units substituted with either β-linked D-glucuronic acid residues at position 2 or β-(1 → 2)-linked D-xylose side-chains, two or three units long, at positions 2 and 4 [see (28)]. The native and deacetylated polysaccharides did not



(28)

cross-react with type III anti-pneumococcal serum, although the Smith-degraded polysaccharide produced a reversal of this effect.[228] Failure to cross-react with the serum was attributed to steric interactions associated with strategically placed D-xylose units in the vicinity of the uronic acid determinants. It was also demonstrated that the stability of these uronic acid residues to periodate oxidation is probably due to the presence of *O*-acetyl groups on position 3. Structural studies on a polysaccharide from *Rhodotorula flava* have revealed the presence of a main chain of (1 → 3)-linked D-mannose residues to which is attached side-chains of (1 → 4)-linked D-xylose residues.[229] A study of the sugar compositions of extracellular polysaccharides produced by strains of *R. glutinis* grown in an acidic medium suggested the existence of at least two kinds of polysaccharides, *viz.* one composed predominantly of D-mannose residues and the other containing L-fucose and D-galactose residues.[230] In a neutral medium, one of the strains produced a polysaccharide containing 94% of L-fucose. Oligogalacturonic acids, from di- to nona-saccharides, have been isolated from a polysaccharide of *S. fragilis* after ion-exchange chromatography.[231] Several unusual oligosaccharides have been isolated from the honeydew of the parasitic fungus *Sphacelia sorghi*; these include

[227] C. G. Fraser, H. J. Jennings, and P. Moyna, *Canad. J. Biochem.*, 1973, **51**, 219.
[228] C. G. Fraser, H. J. Jennings, and P. Moyna, *Canad. J. Biochem.*, 1973, **51**, 225.
[229] G. A. Vitovskaya, N. P. Elinov, N. K. Kozhina, and G. Z. Matov, *Khim. prirod. Soedinenii*, 1972, **1**, 15.
[230] K. Fukagawa, H. Yamaguchi, D. Yonezawa, and S. Murao, *J. Agric. Chem. Soc. (Japan)*, 1973, **47**, 651.
[231] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. (Japan)*, 1972, **46**, 417.

1-*O*-β-D-fructofuranosyl-D-mannitol, 5-*O*-β-D-fructofuranosyl-D-arabinitol, and 1,6-di-β-D-fructofuranosyl-D-mannitol.[232]

Glucans.—Polysaccharides isolated from a number of Basidiomycetes have been shown to inhibit strongly the growth of sarcoma 180 in mice, and the anti-tumour activities were demonstrated to be mediated by the host.[233] It was suggested that certain substances inherent to the host may interact with the anti-tumour polysaccharides, since the higher structure of the polysaccharides is associated with the appearance of activity.[234] All polysaccharides exhibiting tumour inhibition were capable of inactivating the hemolytic activity of the third component of the complement *in vitro*, and some qualitative correlation between the two activities was observed.[235] The interaction of the polysaccharides with serum proteins was studied; it was found that the larger the α-helical structure of the protein, the greater is the anti-tumour effect, suggesting that there might be a close relationship between anti-tumour activity and the α-helical content of the protein.[236] Two polysaccharides showing high anti-tumour activity have been isolated and purified from the edible mushroom *Flammulina velutipes*.[237] One is a D-glucan and the other is composed of D-glucose, D-galactose, D-mannose, and L-arabinose.[238] The polysaccharides of a number of lichens were studied in relation to their host-mediated, anti-tumour activities against sarcoma 180 in mice.[239] A water-insoluble D-glucan from *Evernia prunastri*, containing (1 → 3)- and (1 → 4)-linkages in the ratio of 4 : 1, and a water-soluble D-glucan have been fractionated into an anti-tumour-active polysaccharide, having β-(1 → 3)- and β-(1 → 4)-linkages in the ratio of 3 : 1, and an inactive fraction, having α-(1 → 3)- and α-(1 → 4)-linkages in the ratio of 3 : 2. The linear α-D-glucan isolated from *Acroscyphus sphaerophoroides* was shown to contain (1 → 3)-, (1 → 4)-, and (1 → 6)-linkages. Electron-microscopic and enzymic analyses have shown that the slime track of *Dictyostelium discoideum* represents a two-phase system, with a fibrillar component (most likely cellulose) embedded in an amorphous, protein-containing matrix.[240] The glucan isolated from the cell walls of *Saccharomyces cerevisiae* was found to be heterogeneous; the major component is a branched β-(1 → 3)-glucan (molecular weight 2.4×10^5) containing 3% of β-(1 → 6)-D-glucosidic

[232] R. L. Mower, G. R. Gray, and C. E. Ballou, *Carbohydrate Res.*, 1973, **27**, 119.
[233] Y. Y. Maeda, J. Hamuro, and G. Chihara, *Internat. J. Cancer*, 1971, **8**, 41.
[234] J. Hamuro, Y. Y. Maeda, Y. Arai, F. Fukuoka, and G. Chihara, *Chem. Biol. Interaction*, 1971, **3**, 69.
[235] T. Okuda, Y. Yoshioka, T. Ikekawa, G. Chihara, and K. Nishioka, *Nature New Biol.*, 1972, **238**, 59.
[236] J. Hamuro and G. Chihara, *Nature*, 1973, **245**, 40.
[237] T. Ikekawa, Y. Yoshioka, M. Emori, and F. Fukuoka, *Cancer Chemotherapy Reports*, 1973, **57**, 85.
[238] Y. Yoshioka, T. Sano, and T. Ikekawa, *Chem. and Pharm. Bull. (Japan)*, 1973, **21**, 1772.
[239] T. Takeda, M. Funatsu, S. Shibata, and F. Fukuoka, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2445.
[240] H. R. Hohl and J. Jehli, *Arch. Mikrobiol.*, 1973, **92**, 179.

inter-chain linkages, whereas the minor component is a branched β-(1 → 6)-glucan.[241] A comparison of these results with those reported by others suggested that different glucan preparations may differ in the degree of heterogeneity, and that the major β-(1 → 3)-glucan component may vary considerably in the degree of branching. The minor polysaccharide component was also isolated, and was shown to have a molecular weight of 2.1—2.3 × 10^4, a highly branched structure, and a high proportion of β-(1 → 6)-D-glucosidic linkages.[242] The molecules also contain a small proportion of β-(1 → 3)-D-glucosidic linkages, serving mainly as inter-chain and, possibly, inter-residue linkages. A comparison of the efficiencies of [1-^{14}C]maltose and [U-^{14}C]maltose in the biosynthesis of cell-wall polysaccharides by a strain of *S. cerevisiae* incapable of fermenting maltose has suggested the existence of a reaction that results in the incorporation of the reducing-end unit of maltose into polysaccharides.[243] A number of allergenic D-glucans have been isolated from the mycelia of *Trichophyton rubrum*, *T. mentagrophytes*, and *Microsporum canis* when the organism was grown on surface culture.[244] The presence of α-(1 → 4)-linked D-glucosyl units in these components was demonstrated by enzymolysis. A glycogen-like structure has been proposed for the glucan isolated from *T. rubrum*, based on the identification of oligosaccharides liberated by hydrolysis with α- and β-amylases and pullulanase.[245] At least 80% of the glucan was accounted for by a linear sequence of α-(1 → 4)-linked D-glucose residues joined by α-(1 → 6)-linkages.

Induction of starch production in previously non-amyloid hyphae has been shown to occur only in hyphae of organisms that normally produce some amyloid structures and that have the enzymic systems required to produce starch; only hyphae from fruit bodies contain these enzymes naturally.[246] Nigeran was accumulated intracellularly by *Aspergillus aculeatus* when the organism was grown with a limited supply of nitrogen and at low pH.[247] *De novo* synthesis of nigeran from exogeneous D-glucose accounted for 20% of this sugar transported by the mycelium; cycloheximide inhibited the development of the system for the biosynthesis of nigeran. A method of conformational analysis, based on minimum-energy considerations and allowing four rotatable bonds, has shown that hydrogen bonding is possible in crystalline nigeran between the O-2 and O-3 hydroxy-groups of contiguous residues in α-(1 → 4)-linkage and between the O-2

[241] D. J. Manners, A. J. Masson, and J. C. Patterson, *Biochem. J.*, 1973, **135**, 19.
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[247] M. H. Gold, D. L. Mitzel, and I. H. Segel, *J. Bacteriol.*, 1973, **113**, 856.

and O-4 hydroxy-groups of contiguous residues in α-(1 → 3)-linkage.[248] Nigeran was shown to adopt a 'corrugated ribbon' conformation in the crystal, and an energetically favoured scheme of chain-folding in the polysaccharide was proposed. The composition and structure of the extracellular polysaccharide (pullulan) produced by *Aureobasidium* (*Pullularia*) *pullulans* indicated the existence of a homopolymer of D-glucose containing (1 → 4)-, (1 → 6)-, and (1 → 3)-linkages.[249] The structure of pullulan was shown to be uniform, although the ratio of pullulan to another polysaccharide varied.[250] Evidence for the complete absence of a branched structure accrued from hydrolysis of pullulan by pullulan 4-glucanohydrolase. The composition of the cell-wall polysaccharide of *A. pullulans* has been compared with that of an extracellular polysaccharide; both polysaccharides were found to contain D-glucose, D-mannose, and D-galactose.[251] During the growth of *A. pullulans* there were indications that the elaboration of pullulan and the onset of a morphological change of the organism from a filamentous to a yeast-like form are related to the availability of nitrogen, but not the carbon, in the growth medium.[252] The capsulated yeast *Cryptococcus laurentii* has been shown to produce an extracellular, acidic heteropolysaccharide and, under certain conditions, starch was secreted into the medium.[253] There does not appear to be any connection between the syntheses of the two polysaccharides, and it was concluded that the formation of starch may not be a normal process of the cell. The acidic heteropolysaccharide accumulated in the growth medium only at neutral pH, whereas the starch was formed at low pH.[254] The starch appeared to be structurally different from a polymer of D-glucose comprising the granular, glycogen-like material within the cells. The syntheses of glycogen, cellulose, and acidic polysaccharides during the development of *Dictyostelium discoideum*,[255] and adenylcyclase regulation of the metabolism of glycogen in *Neurospora crassa*,[256] have formed the subjects of other reports.

Mannans.—The D-mannose-containing polysaccharides of yeast have been reviewed.[257] The characteristic type of mannan produced by known species of yeasts has been used as an aid in the identification and classification

[248] P. R. Sundararajan, R. H. Marchessault, G. J. Quigley, and A. Sarko, *J. Amer. Chem. Soc.*, 1973, **95**, 2001.
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[255] M. Sussman, in 'Biochemistry of the Glycosidic linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[256] M. M. Flawia, M. T. Tellez-Iñón, and H. N. Torres, in 'Biochemistry of the Glycosidic linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[257] J. F. T. Spencer and P. A. J. Gorin, *Biotechnol. and Bioeng.*, 1973, **15**, 1.

of yeasts. It was suggested that these polysaccharides could be used instead of plant and bacterial gums as thickeners and dispersing agents, *etc.*

The ^{13}C n.m.r. spectra of oligosaccharides obtained from yeast mannans by partial acetolysis have been shown to exhibit signals at δ_C 93.1—105.4 (corresponding to C-1) and 79.2—81.2 (corresponding to C-2- and/or C-3-substituted D-mannopyranosyl units).[258] Assignments were made on the basis that carbons deshielded by attached D-mannopyranosyl units have resonances at lower field than those of unsubstituted carbons, and by comparisons, made under carefully controlled conditions, of the relative sizes of signals of comparable carbons, which have the same resonances, in members of two homologous series of oligosaccharides. The ^{13}C n.m.r. spectra of certain mannans containing α-(1 → 6)-linked D-mannopyranose main chains could then be rationalized, since they have many signals in common with those of oligosaccharides that contain the structures of the side-chains. A phosphomannan and a glycerol 'teichoic acid' have been isolated from the cells of *Candida intermedia*.[259] The ^1H n.m.r. spectra of metabolic and somatic mannans obtained from extracts of *Ceratacystis stenoceras* and various strains of *Sporothrix schenckii* were found to be superimposable, thus indicating a similarity in chemical structure.[260] The spectroscopic data provided supplementary evidence in favour of the taxonomic identity of these fungi. Purified mannans from six *Pichia* and *Hansenula* sp., having similar ^1H n.m.r. spectra, were differentiated by their ^{13}C n.m.r. spectra into three structural groups; the validity of the technique was borne out by conventional structural determinations.[261] The polysaccharides were found to represent a new structural type having predominantly linear chains of α-(1 → 6)-linked D-mannopyranose units, with (1 → 2)-linked units distributed regularly along the main chain. Degradation of a phosphomannan from *H. holstii* released a phosphorylated pentasaccharide and a phosphorylated core-fragment of high molecular weight that was resistant to further hydrolysis.[262] From studies using chemical, physical, and enzymic methods, the structure (29) was obtained for the pentasaccharide.

α-D-Man*p* 6-phosphate-(1 → 3)-[α-D-Man*p*-(1 → 3)]$_2$-α-D-Man*p*-(1 → 2)-α-D-Man

(29)

A particulate enzyme fraction isolated from *H. holstii* catalysed the transfer of D-mannose from GDP-mannose to endogenous lipid acceptors.[263] Kinetic studies suggested that one of the mannolipids is a precursor of

[258] P. A. J. Gorin, *Canad. J. Chem.*, 1973, **51**, 2375.
[259] I. B. Naumova and G. M. Streshinskaya, *Doklady Akad. Nauk S.S.S.R.*, 1973, **210**, 720.
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[261] P. A. J. Gorin and J. F. T. Spencer, *Canad. J. Microbiol.*, 1972, **18**, 1709.
[262] R. K. Bretthauer, G. J. Kaczorowski, and M. J. Weise, *Biochemistry*, 1973, **12**, 1251.
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cell-wall mannan, since it exhibited characteristics of a polyisoprenyl phosphorylmannose. That the exogenous dolichol phosphate acts as a D-mannosyl acceptor was demonstrated by the conversion of dolichol [^{32}P]phosphate into dolichol [^{32}P]phosphorylmannose.

Wall replication in *Saccharomyces* sp. has been monitored using fluorescein-conjugated concanavalin A; newly synthesized mannan in the mother cells was detected only in the bud scars.[264] The distal tip of the growing bud was identified as the major site of insertion of the new mannan into the existing wall fabric; this area is also known to be the site of deposition of newly synthesized glucan. Thus, with respect to these two principal wall polysaccharides, wall replication in *Saccharomyces* cells resembles the apical mode exhibited by filamentous fungi. Investigations on the biosynthesis of mannan and alkali- and acid-insoluble glucans during cell division showed that both yeast wall components are synthesized continuously throughout the entire cycle.[265] A procedure has been reported for the insolubilization of yeast mannan by entrapment in a polyacrylamide gel.[266] Specific anti-mannan antibodies were separated (by immunosorption) from all other antibodies present in a rabbit antiserum against whole cells of *Candida utilis*. Colloidal gold coated with anti-mannan and anti-non-mannan antibodies was used for ultrastructural visualization of the cell-wall antigens. Two 'wild type' strains of *S. cerevisiae*, having cell-wall mannans characterized by the presence of mannotetraose and manno-phosphorylmannotriose side-chains, respectively, have been hybridized, and the genetic control of mannan structure expressed in the diploid has been investigated.[267] The diploid exhibited the mannan chemotype of the former, rather than an average of the two. A 'dominant' gene was mapped and is considered to be involved in the synthesis of an α-(1 → 3)-mannosyltransferase that adds the terminal α-(1 → 3)-linked D-mannosyl units to the α-(1 → 2)-linked mannotriose side-chains of the mannan. Polymerization of the (1 → 6)-linked backbone was found to be independent of the formation of carrier-bound oligosaccharides. Although this evidence does not rule out a mechanism of biosynthesis similar to that involved in the formation of *Salmonella* O-antigens, it does imply that the mannan is most likely formed by stepwise additions of single D-mannosyl units to the growing chain. A strain of *S. cerevisiae*, which produces mutants defective in the synthesis of cell-wall mannan, was isolated after the initial selection of mutants based on their failure to agglutinate with antiserum specific for the mannotetraose side-chain of the mannan; the immunodominant group of this strain has the structure (30).[268] Two classes of mutant were obtained, one apparently defective in the α-(1 → 3)-mannosyltransferase

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[265] J. M. Sierra, R. Sentandreu, and J. R. Villanueva, *F.E.B.S. Letters*, 1973, **34**, 285.
[266] H. Gerber, M. Horisberger, and H. Bauer, *Infection and Immunity*, 1973, **7**, 487.
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[268] W. C. Raschke, K. A. Kern, C. Antalis, and C. E. Ballou, *J. Biol. Chem.*, 1973, **248**, 4660.

α-D-Man-(1 → 3)-α-D-Man-(1 → 2)-α-D-Man-(1 → 2)-D-Man
(30)

that is presumed to be involved in adding the terminal α-(1 → 3)-D-mannosyl unit to the side-chain. The other class was defective in one of two α-(1 → 2)-mannosyltransferases that are involved in adding the two α-(1 → 2)-linked D-mannosyl units, one of them directly to an α-(1 → 6)-linked D-mannosyl unit of the backbone of the polysaccharide and the other to the first D-mannosyl unit of the side-chain. From this and from complementation studies on diploid crosses of representatives of the different mutant classes it was concluded that all the mutations involve structural genes for the various mannosyltransferases, which are required for mannan synthesis, and not regulatory genes, which control expression of a particular mannan chemotype. Various mutant strains of *S. cerevisiae* have been analysed for their ability to bind Alcian Blue.[269] It was shown that the ability to bind this dye is directly related to the presence of mannosyl phosphate groups on the surface of the cell or in the cell wall. A comparison of yeast-mannan mutants by electron microscopy showed that the mutations do not result in changes in either the thickness of the cell-wall or surface characteristics.[270]

A sterol-binding polysaccharide isolated from *S. cerevisiae* was identified as a cell-wall mannan.[271] The binding of sterols was shown to be a biphasic function of the concentration of mannan and to be independent of pH over a measured range. The lipid-linked sugars in *S. cerevisiae* have been suggested to be involved not only in the transfer of mannosyltransferases, but also in the process of formation of mannan.[272] The results have pointed to a more general role of polyprenol phosphate sugars in yeast and to a relative unspecificity of the enzyme–lipid-linked sugars with respect to the type of lipid acceptor.

Chitin.—Chemically extracted walls of seven species of *Oomycetes* were found to contain small amounts of 2-amino-2-deoxy-D-glucose, possibly originating from chitin.[273] Species belonging to the order *Peronosporales* appeared to contain less amino-sugar than the *Saprolegniales*. Chemical and ultrastructural studies on isolated cell walls of *Epidermophyton floccosum* have indicated the presence of chitin.[274] A rapid, chemical estimation of chitin in filamentous fungi in plant tissues has been based on deacylation of chitin to chitosan with alkali, followed by deamination with nitrous acid and colorimetric determination of the resulting 2,5-anhydro-D-mannose with 3-methyl-2-benzothiazolone hydrazone.[275]

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[275] J. P. Ride and R. B. Drysdale, *Physiol. Plant Pathol.*, 1972, **2**, 7.

The chitin-synthetase activity of a 'microsomal' fraction from *Mucor rouxii* was found to increase during storage under certain conditions, but the activity could be blocked by an inhibitor present in the soluble cytoplasm.[276] These observations are in accord with a former proposal concerning the mechanism of control of chitin synthetase in *Saccharomyces* (see E. Cabib and V. Farkas, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2052), which suggested that the enzyme is produced in a zymogen state and is then converted into its active form by proteolysis.

Preparations rich in bud scars obtained from the walls of *S. cerevisiae* have been studied by *X*-ray diffraction and fluorescent-dye techniques.[277] The emergence of buds was found to be preceded by the appearance of stainable structures, with an increase in the content of 2-amino-2-deoxy-D-glucose linked with the appearance of the annular structure. The morphological basis of budding was discussed with special reference to the role of the annular structure.

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5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

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Introduction

Processes of transcription of the sequence of bases in the genome, followed by translation of messenger RNA, lead to the production of a polypeptide chain which may undergo glycosylation as a post-translational event. Thus, most of the glycosaminoglycans, with the possible exception of hyaluronic acid, may be considered as one group of glycoproteins.[1, 2] The possibility that liver glycogen [1, 3, 4] and potato starch [5] require polypeptide backbones as their initiators, and are, therefore, glycoproteins, needs further investigation.

Examination of the distribution of radioactivity in the serum glycoproteins of rats injected with L-[^{14}C]asparagine or L-[^{14}C]aspartic acid has led to the conclusion that the L-asparagine residue is the major, if not the only, site of glycosylation leading to the formation of carbohydrate–peptide (D-Glc*N*Ac-Asn) linkages involving a substituted asparagine residue.[6] Dicoumarol may inhibit the production of this type of linkage in prothrombin for patients receiving this drug, as well as reducing the rate of addition of the rest of the sugars to the carbohydrate moieties of this glycoprotein.[7]

Triplet amino-acid sequons of the type -Asn-*X*-Thr(or Ser)-, needed for the glycosylation of asparagine to occur,[1] have been found to include those in which *X* is an aromatic amino-acid residue, since a glycopeptide of the form -Asn(Carb)-Tyr-Ser- has been isolated from the α-amylase of *Aspergillus oryzae*.[8] There have been earlier tentative suggestions [9] that phenylalanine and tryptophan occupy analogous positions in two human myeloma K-type light-chains.

Glycosylated hydroxylysine residues in the collagen of cuttlefish skin and in glycopeptides prepared from the Cuvierian tubules of the sea

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[2] R. G. Spiro, *Adv. Protein Chem.*, 1973, **27**, 350.
[3] C. R. Krisman, *Ann. New York Acad. Sci.*, 1973, **210**, 81.
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[9] H. C. Sox and L. Hood, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **66**, 975.

cucumber *Holothuria forskali*[10] are present in sequences of the form -Gly-*X*-Hyl-Gly-*Y*-Arg-, as in a number of other collagens. The final arginine residue is probably not involved as a marker for the glycosylation reaction, since glycopeptides isolated from skin-collagen α_2-chain[11] and cattle α_1-chain[12] have leucine and threonine residues, respectively, in the same relative position. Moreover, one of the glycopeptides from the sea cucumber *Stichopus japonicus* has been reported (without details) to have a different type of amino-acid residue at this position.[13] Thus, the sequence of amino-acids recognized by the galactosyltransferase may well be a tri- or a tetra-peptide.

D-Xylosyl-L-serine (SER) residues in chondroitin sulphate from bovine nasal cartilage occur in the sequences SER-Gly, Ala-SER-Gly, Gly-Ala-SER-Gly, and Leu-SER-Gly.[14]

The sequon for glycosylation of a threonine residue by 2-acetamido-2-deoxy-D-galactose was reported to be of the form Thr-*X*-*X*-Pro,[15] rather than Pro-Thr[16] or Thr-*X*-Pro[17] or Pro-Thr-*X*-Pro.[18] On the basis of the first report, it was suggested that it is residue number 26 or 28 of cattle casein glycopeptide that carries the carbohydrate moiety Neu*N*Ac-(2 → 3(6))-D-Gal-(1 → 3)-D-Gal*N*Ac,[19] with further substitution of the D-galactose and/or the 2-acetamido-2-deoxy-D-galactose residues by additional D-galactose residues.[20] However, exceptions are known to all the sequons proposed. Moreover, analytical results for a number of glycoproteins, including those from the submaxillary glands of cattle, sheep, and pig, human gastroferrin and gastric mucin, ovarian cyst blood-group substances, and colloid carcinoma mucin clearly show that there cannot be a general sequon involving equal numbers of threonine or serine residues on the one hand and proline residues on the other.

The mechanisms involved in the formation of animal glycoproteins have been reviewed.[21] The *in vitro* reactions have been found to proceed often without involvement of either dolichol phosphate or other lipid derivatives of sugars as intermediates,[22] although substances of this type are formed in

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liver [23–26] and appear to be capable of acting as donors of D-mannose to protein.[27] A mannolipid also appears to act as the sugar-donor in the formation, by a plasma-cell tumour, of a K-type myeloma glycoprotein, with initial transfer of D-mannose from GDP-mannose.[28] Similar observations have been made concerning glycoprotein biosynthesis with extracts from hen oviduct and bovine thyroid.[29] It is not clear whether the retinol mannolipid, which can be formed by liver, plays any direct role in the biosynthesis of glycoproteins.[30] The stimulation by CDP-choline of the incorporation of 2-acetamido-2-deoxy-D-glucose into endogenous acceptors of liver by rough microsomes [31] may result from a detergent-like action.[32] A sugar–lipid intermediate may also be involved in this reaction.[33]

Knowledge is developing concerning the chemical pathology of glycoproteins, and it is on the degradative pathways that enzyme defects are being revealed. The inborn error of metabolism in which there are reduced levels of the enzyme (E.C. 3.5.1.26) that cleaves D-Glc*N*Ac-Asn-type linkages (provided the α-amino- and α-carboxy-groups are unsubstituted) is quite common in some areas of the world.[34, 35] The high levels of D-Glc*N*Ac-Asn found in diseased tissues do not appear to have much effect on either the rate or extent of formation of L-asparaginyl-tRNA.[36]

Various analytical procedures for glycoproteins have been summarized.[37] Estimation of the sialic acid content of glycoproteins containing *O*-acetylated sialyl residues needs special care, since *O*-acetyl residues, at least in some positions (C-7 and/or C-8), are the cause of reduced rates of cleavage of α-D-sialyl linkages in acid solutions.[38] A method of estimating either bound or free sialic acid, by determining the u.v. absorption of the complex formed between the sugar and *o*-phenanthroline, has been applied to fetuin and to ovomucoid.[39] Assays carried out at low concentrations of fetuin gave values agreeing with those obtained in earlier work, but the amounts reported for ovomucoid

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(3.5%) were larger than those usually found. The molecular weights of some glycoproteins may be estimated, under appropriate conditions and with a fair degree of accuracy, by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulphate.[40, 41]

A number of reviews have appeared recently on general aspects of glycoproteins;[1, 2, 42, 43] on the use of α-mannosidase as a structural reagent for these macromolecules;[44] and on the carbohydrate moieties present in many transplantation antigens,[45] in many enzymes,[46] and on the surface of animal cells.[47–51] Reviews covering more specific areas are mentioned later in this Report.

Microbial Glycoproteins

A β-D-glucosidase (molecular weight 4.1×10^4) secreted by *Aspergillus fumigatus* has been shown to contain about 17 mol of D-mannose and 2 mol of 2-amino-2-deoxy-D-glucose per mole, distributed as two carbohydrate moieties linked by D-Glc*N*Ac-Asn linkages to the polypeptide chain.[52]

The glycoprotein, reticulum, of the hyphal cell wall of *Neurospora crassa* has been isolated as a glycopeptide complex by extraction with ammonium hydroxide. It contains D-galactosyl–serine linkages.[53] Dolichol monophosphate-D-mannose is an intermediate in the production of the glycoprotein yeast mannan, as well as in the formation of the cell-wall.[54] It is probably specifically involved in linking the mannan to serine and/or threonine residues.[54]

Calcium-binding glycoproteins have been shown to be present on the membranes of some water-moulds. The binding of calcium ions is allosterically regulated by cytokinins,[55] which also activate the transport of calcium ions into the cell.[56]

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The role of the protein moiety of the lipopolysaccharide-protein present in the cell envelope of *Escherichia coli* in the biosynthesis of the macromolecule is under investigation.[57]

The *exo*-β-2-acetamido-2-deoxyglucosidase released into the medium by *Bacillus subtilis* B may be involved in glycoprotein catabolism.[58] Production of the enzyme is de-repressed during gluconeogenesis and the initial stages of sporulation.[59]

The protein comprising the gas vesicles of *Halobacterium halobium* contains D-galactose, which is released on acid hydrolysis (1.67M-sulphuric acid for 3 hours at 100 °C), but the nature of the linkage is unknown.[60] The cell wall of the green alga *Chlamydomonas reinhardii* contains glycoproteins having hydroxyproline, arabinose, and galactose among their constituents;[61] galactose is linked to hydroxyproline.[62]

Oral bacteria appear to produce enzymes capable of cleaving the carbohydrate moieties from salivary amylase, thereby converting it into simple proteins.[63]

Higher Plant Glycoproteins

Many of the lectins fall under this heading; they are considered in the following section. A review dealing with a number of aspects of higher plant glycoproteins has appeared recently.[64]

Degradation of the cell wall of sycamore by hydrazinolysis has led to the production of soluble glycopeptides, the predominant one of which contains hydroxyproline, arabinose, and galactose only, in the molar ratios 2 : 7 : 11, with each amino-acid residue carrying a sugar moiety. The dipeptide portion is cyclic (4-*trans*-hydroxy-L-prolyl)-4-*trans*-hydroxy-L-proline.[65] D-Galactose also appears in other positions in extensin from cell walls of the tomato plant. Each serine residue in the polypeptide sequence Ser-Hyp-Hyp-Hyp-Hyp-Ser-Hyp-Lys- is substituted by a D-galactose residue, and the carbohydrate–peptide linkages in the intact protein are labile under alkaline conditions.[66] A D-galactose–hydroxyproline linkage has been reported to occur in an arabinogalactan–protein isolated from wheat endosperm.[67] Glycoproteins containing hydroxyproline, arabinose, and galactose have also been found in the cell walls of apple fruit.[68]

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Cells of sycamore and tobacco calluses grown in culture rapidly incorporated 2-amino-2-deoxy-D-glucose, mainly into cell-wall glycoproteins. In corn roots, however, incorporation occurred predominantly into cytoplasmic glycoproteins.[69] Plant cells grown in suspension culture are known to release certain cell-wall components into the medium, and one of those released by soybean is a glycoprotein (36% protein) containing large amounts of galacturonic acid (46%) and lesser amounts of arabinose.[70]

The amount of glycoprotein II present in kidney beans, characterized by Pusztai and Watt,[71] was found to increase most rapidly as the coats of growing bean seeds turn grey; this rate of increase is five times greater than the rate of increase of total protein.[72]

Lectins

Lectins are widely used in studies of animal-cell surfaces, and many properties of these substances, including their interactions with animal cells, have been reviewed,[73, 74] as has the nature of the receptor sites for phytoagglutinins on the human-cell surface.[75] Comparative studies have also been made on the anti-A lectins from various snails (*Helix pomatia* and *Helix aspersa*).[76]

Lectins isolated from the lobster (*Panulirus argus*) and the oyster (*Crassostrea virginica*) are glycoproteins,[77] and there are a number of lectins possessing different specificities in the snail *Pomacea urceus* protectin.[78] Proteins able to bind D-glucose have been found in mammalian brain [79] and kidney,[80, 81] but their functions are unknown.

Abrin, which binds D-galactose residues, has been shown to be composed of four identical, or at least very similar, subunits, each of which is made up of two glycopeptide chains of molecular weights 3.5×10^4 and 3×10^4. *X*-Ray diffraction studies have shown that the molecule contains at least one exact, or near-exact, two-fold axis relating pairs of monomers.[82]

The tetrameric form of concanavalin A consists largely of chains arranged in two anti-parallel sheets (or β-structures) without regions of

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α-helix. Binding sites for Mn^{2+} and Ca^{2+} ions are some distance (20 Å) from the binding site for carbohydrate, which consists of a deep pocket lined with hydrophobic residues.[82a, 83] The binding site for Mn^{2+} ions (S_1), at least in the dimeric form of the lectin, will also bind Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} ions in decreasing order of affinity.[84] Gd^{2+} and Tb^{3+} ions will also bind at site S_1, as well as at the site (S_2) for Ca^{2+} ions.[85]

Partial purification of human α_1-antitrypsin – an inherited deficiency of which can lead to pulmonary emphysema and liver cirrhosis – has been achieved by affinity chromatography utilizing concanavalin A covalently bound to Sepharose 4B.[86, 87] A similar chromatographic procedure has been used to separate human urinary erythropoietin from granulocyte-colony-stimulating glycoprotein, with the latter adhering to the affinity matrix.[88] Glycoproteins from the glycolipoprotein envelopes of three strains of influenza virus and of the Sendai virus have been separated by chromatography on an affinity adsorbent composed of Sepharose 4B substituted by the lectin from *Lens culinaris*; the detergent sodium deoxycholate was used throughout the chromatographic procedure.[89] Concanavalin A–Sepharose was also used to purify the phytoagglutinins from lima bean, wax bean, soybean, and *Bandeiraea simplicifolia*.[90] Wheat-germ agglutinin did not bind to the column,[90] as might have been anticipated from studies which showed this lectin not to be a glycoprotein.[91, 92]

Wheat-germ agglutinin has been purified by binding it to a support (formed by linking 6-amino-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside to Sepharose 4B) and then eluting with solutions of 2-acetamido-2-deoxy-D-glucose.[93] Haemagglutinins from the seeds of *Sophora japonica* can also be purified by affinity chromatography, either by binding directly to Sepharose 6B and eluting with solutions of D-galactose,[94] or by utilizing an insoluble support composed of polyleucine and pig gastric mucin.[95] The relationship between the products obtained by these procedures will be of interest. The toxic protein, ricin, from the castor bean, which acts on the 60*S* ribosomal subunit,[96] may be separated from the lectin in the beans by affinity chromatography on Sepharose 4B, since ricin does not bind.[97]

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An immobilized form of chicken-brain arylsulphatase has been prepared by allowing it to interact with concanavalin A.[98]

The mechanisms of the effects of lectins on lymphocyte transformation are not well understood. Blastogenesis may also be induced in cultured, mouse-spleen cells by the action of galactose oxidase, provided that the cells have been treated first with neuraminidase.[99] These findings suggest that treatment with neuraminidase exposes D-galactose or 2-acetamido-2-deoxy-D-galactose residues within the membrane, and that it is the oxidation of these residues that triggers off blastogenesis. Transformation can also be induced in the modified, but not original, cells by the action of soybean lectin.[100]

Selective activation of thymus-derived lymphocytes by concanavalin A has been described.[101] Interferon inhibited the transformation of mouse-spleen cells induced by concanavalin A,[102] and commercial preparations of human chorionic gonadotrophin and somatomammotrophin inhibited the phytohaemagglutinin-induced blastogenesis of human lymphocytes.[103]

The lectin from the red kidney-bean phytohaemagglutinin that stimulates the production of RNA in lymphocytes has been found to differ from the factor in the bean that can cause increases in rate of the formation of RNA in *Escherichia coli*.[104] Formation of ornithine decarboxylase preceded initiation of DNA synthesis in the stimulated lymphocytes.[105] Calcium ions do not appear to be needed for interaction of the phytohaemagglutinin with the carbohydrate moieties of the lymphocyte surface, but they are needed for the transport of amino-acids.[106]

Transformation of cultured rat lymph-node cells by concanavalin A may be inhibited by complement under certain conditions. The probable sequential binding of concanavalin A and the complement to the cell surface may have important implications for studies of cell-population homeostasis.[107]

Loss of concanavalin A from the cell membranes of lymphocytes stimulated by the lectin has been shown to lead to the rapid appearance of new receptor sites.[108] It is not known whether the lectin is shed from the cell surface by pinocytosis or by elution,[108] nor whether the lectin is shed together with its binding site, in which case a new receptor site would be rapidly synthesized.[108, 109]

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Concanavalin A, used to stimulate human peripheral blood lymphocytes, became largely irreversibly bound after twenty hours. It was suggested to be intracellular and to have lost its ability to stimulate.[110] Lectins, in any case, do not cause an irreversible de-repression leading to repetitive cell division.[111]

Cultured spleen and thymus cells from a 35-day-old guinea-pig foetus were found to be responsive to phytohaemagglutinin, but no age-related differences were observed in the effect of the lectin throughout gestation.[112] On the other hand, there is a diminished response to the lectin in spleen cells from ageing mice.[113]

There appear to be at least two target cells for concanavalin A in mouse spleen: one on which the antigen normally acts and one that undergoes increased incorporation of thymidine.[114] An increased response to plaque-forming cells was found for cultured mouse-spleen cells that had been treated with submitogenic levels of lectins or that had been derived from mice previously injected with concanavalin A. Larger doses were found to be inhibitory.[115]

Protection against trypsin was afforded by concanavalin A to certain glycoproteins in the cell surface of cultured mouse L-cells.[116] This finding could be of considerable value in the development of procedures for studying glycoproteins of cell surfaces.

Low concentrations of wheat-germ agglutinin and concanavalin A can, like insulin, enhance the rate of transport of D-glucose and inhibit lipolysis stimulated by epinephrine in isolated adipocytes.[117] The lectins bind to the insulin receptors of fat and to liver-cell membranes, which appear to be glycoproteins.[118]

The greater agglutinability of tumour cells, compared with cultured, normal cells, is fairly well established. Cells derived from acute lymphoblastic leukaemias were found to be more readily agglutinated by the wheat-germ lectin than were normal cells.[119] Virus-transformed cells grown in culture bound considerably more lectin than did their non-transformed counterparts.[120] The distribution of concanavalin A on cultured mouse 3T3 cells, trypsinized cells, or those transformed with the polyoma virus was shown to be essentially uniform, provided the cells were fixed before adding the lectin. Subsequent to the binding of concanavalin A in unfixed

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cells, there is an induced redistribution of the binding sites.[121] Similar changes in the relative positions of the glycoprotein-acceptor sites occurred with synaptosome membranes.[122] Wheat-germ agglutinin was shown to bind to a di-*N*-acetylchitobiosyl-asparaginyl entity in a number of tumour-cell surfaces.[123]

Partial purification from the surface membrane of a cultured line of mouse leukaemia cells of the receptor for wheat-germ lectin has been effected.[124] The material did not react with concanavalin A, which is able to agglutinate the whole cells.[125] It is likely that the receptors on the leukaemia cells for the two lectins used are quite distinct, both chemically and topographically.

It was shown that certain cultured tumour or transformed cells become more malignant in animals if the cells are first treated with concanavalin A.[126] These results appear to be in contrast to others, which showed a reduced tumorigenicity of transformed cells when animals were injected intraperitoneally with concanavalin A.[127]

Changes in the cell surface, which can be measured by an increased agglutinability with lectins, have been correlated with escape from density-dependent growth control and with malignancy; this area has been reviewed recently.[128] One attempt to examine this hypothesis involved treating density-inhibited mouse 3T3 fibroblasts with pronase in order to reveal more binding sites for concanavalin A, but no increase in the number of cells occurred.[129] The binding of univalent concanavalin A to neural retinal cells, which had been trypsinized, did not alter their rate of reaggregation, nor did it alter the sorting phenomenon in mixed cell populations.[130]

Concanavalin A has been shown to bind to rat-liver mitochondria by a mechanism involving glycoproteins. [^{3}H]Acetylconcanavalin was used to measure the degree of binding; at low concentrations this is about as effective as a mitogen or as the unmodified form,[131] and at high concentrations, acetylated and succinylated concanavalin A are bound more effectively.[132] Gel-filtration studies of the solubilized (1% Triton X-100) receptors indicated molecular weights of $4—8 \times 10^4$.[133]

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[131] C. F. Reichert, P. M. Pan, K. P. Mathews, and I. J. Goldstein, *Nature New Biol.*, 1973, **242**, 146.
[132] G. R. Gunther, J. L. Wang, I. Yahara, B. A. Cunningham, and G. M. Edelman, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 1012.
[133] R. H. Glen, S. C. Kayman, and M. S. Kuhlenschmidt, *J. Biol. Chem.*, 1973, **248**, 3137.

The use of a lectin from *Lotus tetragonolobus* has led to the discovery of a previously unknown human blood-group antigen, closely related to the H and Le^b antigens; it is present in saliva and in red cells of secretors and non-secretors alike, but is absent in Bombay individuals.[134] Its synthesis, like that of A and B substances, is probably under the control of the H gene.

The B-erythrocyte receptor site for pea lectin has been found to reside in a glycopeptide possessing approximate molar ratios for D-galactose, D-mannose, L-fucose, 2-amino-2-deoxy-D-glucose, *N*-acetyl-neuraminic acid, aspartic acid, and serine of 4 : 2 : 1 : 8 : 1 : 1 : 1, respectively.[135] It underwent cross-reaction with B-erythrocyte–anti-B serum, and interacted with concanavalin A and lentil lectin. The binding activity of the pea lectin has been shown to depend on certain tryptophanyl residues within the protein.[136] The anti-B lectin produced by cultures of *Streptomyces* was found to bind to sugars with the D-galactopyranose configuration at C-2 and C-4.[137]

The erythrocyte receptor site for *Robinia* lectin has been shown to contain the structure (1); the receptor-determinant sugars are predominantly

$$\begin{array}{r} \beta\text{-D-Gal-}(1 \rightarrow 3 \text{ or } 4)\text{-}\beta\text{-D-Glc}N\text{Ac-}(1 \rightarrow ?)\text{-D-Glc}N\text{Ac-Asn} \\ \alpha\text{-Neu}N\text{Ac-}(2 \rightarrow 6)\text{-}\beta\text{-D-Gal-}(1 \rightarrow 3 \text{ or } 4)\text{-}\beta\text{-D-Glc}N\text{Ac-}(1 \rightarrow 2)\text{-}\alpha\text{-D-Man-} \\ (1 \rightarrow 2)\text{-D-Man} \end{array}$$

(1)

the outer D-galactose and inner D-mannose residues.[138] It is perhaps relevant to mention that internal 2-*O*-linked D-mannopyranosyl residues in glycoproteins are frequently bound also by concanavalin A.[139] The binding of internal sugar residues by lectins confirms earlier studies.[140]

It was inferred from binding experiments with concanavalin A that maturation of Ascidian eggs is accompanied by rearrangement of glycoproteins in the cell surface.[141]

The purification and properties of lectins from wheat germ,[91] potatoes,[142] and seeds of *Phaseolus coccineus*[143] and rice (*Oryza sativa* L.)[144] have been described. Isolectins from wax beans were purified and shown to be glycoproteins.[145] The growth inhibitor for rats that is present in wax beans appears to be a haemagglutinin that also stimulates lymphocyte transformation.[146]

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Potato lectin contains about 50% arabinose, and there is probably a linkage between this sugar and hydroxyproline. The glycoprotein bound oligomers of β-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucose.[142] Rice-seed lectin contains only about seventy amino-acid residues and, it is reported, large amounts (thirteen residues) of D-glucose and smaller amounts of other sugars.[144] A lectin from kidney beans, which agglutinates leucocytes but not erythrocytes, has been crystallized. It is a glycoprotein (molecular weight 1.26×10^5) containing eighteen and fifty-seven residues of 2-amino-2-deoxy-D-glucose and D-mannose, respectively, per mole.[147] The lectin exhibits slight immunosuppression, but has a high toxicity, so its clinical use for prolonging graft-survival is unlikely.[148]

Concanavalin A markedly reduced the extent of binding of bacteriophage K to the walls of a number of Gram-positive bacteria; the teichoic acids in the cell walls contain α- but not β-linked 2-acetamido-2-deoxy-D-glucose residues.[149] Certain Gram-negative bacilli also agglutinated with concanavalin A, and the relation of this reaction to the somatic antigens of *Salmonella* serotypes was explored.[150]

The fluorescence of 4-methylumbelliferyl α-D-mannopyranoside is quenched on binding to concanavalin A; this finding was exploited in the development of a method that revealed that association depends on a group with a pK_a of 3.5.[151] Measurements were also made of the binding by concanavalin A of *p*-nitrophenyl α-D-mannopyranoside,[152] and of a variety of *p*-substituted-phenyl glycosides.[153] Concanavalin A inhibited the hydrolysis, catalysed by rat-liver lysosomes, of *p*-nitrophenyl α-D-glucopyranoside and -mannopyranoside.[154]

The lectin from the castor bean (*Ricinus communis*) precipitated on treatment with a series of branched-chain galactomannans, but not with unbranched galactans.[155]

Blood-group Substances

The human blood-group antigens[156] and the enzymic basis for the ABO and Lewis groups[157] have been discussed. An enzyme preparation from

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[152] R. D. Gray and R. H. Glew, *J. Biol. Chem.*, 1973, **248**, 7547.
[153] F. G. Loontiens, J. P. Van Wauwe, R. De Gussem, and C. K. De Bruyne, *Carbohydrate Res.*, 1973, **20**, 51.
[154] E. Bourque and J. Kanfer, *Life Sciences Part II*, 1972, **11**, 1199.
[155] J. P. Van Wauwe, F. G. Loontiens, and C. K. De Bruyne, *Biochim. Biophys. Acta*, 1973, **313**, 99.
[156] B. Boettcher, in 'Biochemical Genetics of Man', ed. D. J. H. Brock and O. Mayo, Academic Press, New York, 1972; E. A. Kabat, in 'Carbohydrates in Solution', Advances in Chemistry Series, American Chemical Society, 1973, Vol. 117, p. 334.
[157] V. Ginsburg, *Adv. Enzymol.*, 1972, **36**, 131.

human milk with lactose synthetase activity transferred D-galactose from UDP-D-galactose to an acceptor that had been prepared from blood-group H substance by sequential removal of terminal L-fucose and D-galactose residues. 'Precursor substance', which underwent cross-reaction with type 14 *Pneumococcus* anti-serum, was formed.[158] A GDP-L-fucose:glycoprotein glycosyltransferase has been detected in sera of group O individuals.[159] The effects of differing growth conditions upon the expression of blood-group H activity of epithelioid cells[160] may become better understood when an L-fucosyltransferase has been isolated.

Blood-group A_2 erythrocytes may have fewer A-specific sites than A_1 cells. An enzyme preparation from gastric mucosa of A_1 individuals can catalyse the conversion of O-erythrocytes first into cells with A_2 reactivity and then, after longer periods, into A_1 cells.[161] Other studies have demonstrated that 2-acetamido-2-deoxy-D-galactosyltransferases in the sera of A_1 and A_2 individuals differ qualitatively.[162] Adult bone marrow of rabbits has been shown to contain an α-D-galactosyltransferase that catalyses the formation *in vitro* of blood-group B-specific pentaglycosylceramide.[163]

A glycoprotein (unit molecular weight 5.3×10^4) containing 55% carbohydrate has been isolated from human O-erythrocyte membranes. It carries H and MN blood-group activities, which reside in alkali-labile carbohydrate moieties, as well as receptor sites for a number of plant lectins. The heterosaccharides for the latter appear to be attached to the polypeptide chain by linkages that are relatively stable to alkali.[164] Linkages of this type have also been reported between carbohydrate moieties containing the A, B, and H activities in glycoproteins isolated from the same type of membrane,[165] a result in contrast with that just described and also with that found earlier by Marchesi. However, the nature of the linkage was not identified.

Small, reduced polysaccharides (13—15 residues) have been obtained from a number of ovarian-cyst H and Le^a blood-group substances after treatment with alkaline borohydride.[166] Reduced oligosaccharides, with structures not previously described, were isolated from an Le^a- and from H- and Le^b-active substances after similar degradation.[167] The oligosaccharides were tested as inhibitors in five different antibody or lectin systems.[168]

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[159] J. R. Munro and H. Schachter, *Arch. Biochem. Biophys.*, 1973, **156**, 534.
[160] W. J. Kuhns and C. Pann, *Nature New Biol.*, 1973, **245**, 217.
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[167] L. Rovis, B. Anderson, E. A. Kabat, F. Gruezo, and J. Kiao, *Biochemistry*, 1973, **12**, 5340.
[168] L. Rovis, E. A. Kabat, M. E. A. Pereira, and T. Feizi, *Biochemistry*, 1973, **12**, 5355.

α-L-Fuc
1
2
α-D-GalNAc-(1 → 3)-β-D-Gal-(1 → 3 or 4)-β-D-GlcNAc-(1 → 4)-β-D-Gal
1
3 or 6
β-D-Gal-(1 → 4)-D-GlcNAc-(1 → 4(?))-D-GlcNAc-(1 → 3)-D-Gal-
6 or 3
6-O-S
(1 → 3 or 4)-D-GalNAc-serine (threonine)
α-L-Fuc
1
2
β-D-Gal-(1 → 3 or 4)-β-D-GlcNAc-(1 → 4)-β-D-Gal
1
(2)

An enzyme present in filtrates of *Clostridium perfringens* cultures has been found to cleave 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose from a variety of glycoproteins [pig submaxillary (A, H, and In) mucins, pig gastric (A and H) mucins, cow colostrum, and others]. The disaccharide may well arise from the region immediately adjacent to the serine (or threonine) residues of the polypeptide chains.[169] Penta-, tetra-, tri-, and di-saccharides have been isolated from acetolysates of sulphated blood-group-active (A + H) glycoproteins from pig gastric mucin; on the assumption that these all arise from a single, carbohydrate, prosthetic group, structure (2) is indicated for the mucin.[170]

Digestion by pronase of blood-group substances from ovarian cysts resulted in loss of amino-acids, but little loss of carbohydrate. The molar compositions of amino-acids of the macromolecular products were very similar to those of the starting products, and the results suggested that there are regions of the polypeptide chain devoid of sugar residues.[171] Small glycoamino-acids and glycopeptides, whose structures are Thr(D-Gal*N*Ac)-Ala, Ser(α-D-Gal*N*Ac), and Thr[D-Gal*N*Ac-(1 → 3 or 4)-D-Gal*N*Ac]Ala, have been isolated from blood-group substances.[172, 173] Blood-group substance B, isolated from horse-stomach lining, contains serine and threonine residues linked to 2-acetamido-2-deoxy-D-galactose residues, which act as branch points.[174, 175]

From studies on identical twins, it appears that the amount of A substance determined serologically in saliva is greater in A_1 than in A_2 individuals, and that the amounts of A and B substances are greater in Lewis-negative individuals. The known structures of the carbohydrate moieties leading to A, B, H, and Lewis activities might lead one to expect that the concentration of the blood-group substances, as measured serologically, is closely regulated genetically.[176]

The cellular A-like antigen (Tr) in dogs appears to be formed independently of H substance, since the latter does not occur in red cells of the species.[177] If Tr antigen is closely related structurally to A antigen, the results suggest that the 2-acetamido-2-deoxy-D-galactosyltransferase needed to produce it must have different receptor requirements from those needed by the A-forming enzyme present in man.

[169] C. C. Huang and D. Aminoff, *J. Biol. Chem.*, 1972, **247**, 6737.
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[171] A. S. R. Donald, *Biochim. Biophys. Acta*, 1973, **317**, 420.
[172] N. K. Kochetkov, V. A. Derevitskaya, L. M. Likhosherstov, and S. A. Medvedev, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 748.
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[174] V. A. Derevitskaya, L. M. Likhosherstov, N. P. Arbatsky, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2782.
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[176] P. Sturgeon, E. Bates, and D. McQuistan, *Vox Sanguinis*, 1973, **25**, 52.
[177] A. J. Bowdler, R. W. Bull, C. Dries, R. Slating, and S. N. Swisher, *Vox Sanguinis*, 1973, **24**, 228.

Further studies have been made on antigens common to human and bacterial cells. Sepsis due to blood-group B-active *Proteus mirabilis* was found to be accompanied by the production of A-active sites on the *Proteus*, which was probably catalysed by the patient's red-cell stroma. Antibodies formed were directed against not only the invasive organism, but also against the patient's A-type red cells.[178]

Fourteen types of pneumococcal polysaccharide have been individually coated on to type-O red cells previously treated with chromic chloride. These were then used in assessing antibody titres in individuals immunized with the polysaccharides, to form the basis of a simple, reproducible method of assay.[179]

Serological studies on the salivary glycoproteins of individuals with the red-cell phenotype Le (a − b − x −) have led to the suggestion that the *le* gene may be an inefficient *Le* gene. The fucosyltransferase, the product of gene expression, was not examined, nor was a chemical examination made of the salivary glycoproteins.[180]

It has been shown serologically that I, H, and HI substances are present on red cells and in other body tissues, but not in the Rhesus monkey (apart from H substance, which is present in extracts of gastric mucosa).[181] These types of antigens, at least on human cells, were relatively stable in the cold on red cells that had been collected as clotted blood.[182] At least six types of I specificity are present on certain precursors of A, B, H, and Lewis substances. The precipitin reaction between an anti-I serum and a precursor substance was best inhibited by oligosaccharides with the non-reducing, terminal structure β-D-Gal-(1 → 4)-β-D-Glc*N*Ac-(1 → 6); this structure is known to occur in the precursor.[183]

Blood-group P_1 substance from hydatid cyst fluid is a glycoprotein (68% carbohydrate) with an amino-acid composition similar to that of A, B, H, and Lewis substances. Prolonged treatment of the substance with coffee-bean α-galactosidase has been reported[184] to lead to a reduction in P_1-inhibitory activity, a finding observed only when a more sensitive haemagglutination technique than that previously employed[185] was used.

α-D-Galactopyranosyl groups have been confirmed as the immuno-determinant groups of P^k antigenicity.[186]

A new blood-group antigen, Hov, which is inherited as a dominant Mendelian character, has been found in three families.[187]

[178] G. W. Drach, W. P. Reed, and R. C. Williams, *J. Lab. Clin. Med.*, 1973, **81**, 919.
[179] A. J. Ammann and R. J. Pelger, *Appl. Microbiol.*, 1972, **24**, 679.
[180] M. B. Arcilla and P. Sturgeon, *Vox Sanguinis*, 1973, **25**, 72.
[181] R. M. Lambert and S. K. Zelenski, *Vox Sanguinis*, 1973, **25**, 182.
[182] R. M. Lambert, J. P. Downing, and S. K. Zelenski, *Vox Sanguinis*, 1973, **24**, 362.
[183] T. Feizi and E. A. Kabat, *J. Exp. Med.*, 1972, **135**, 1247.
[184] D. J. Anstee and G. I. Pardoe, *European J. Biochem.*, 1973, **39**, 149.
[185] W. T. J. Morgan and W. M. Watkins, in 'Proceedings 9th International Congress Internat. Soc. Blood Transf.', Karger, Basle, 1974, p. 225.
[186] D. Voak, D. Anstee, and G. I. Pardoe, *Vox Sanguinis*, 1973, **25**, 263.
[187] A. Szaloky, N. K. Sijpesteijn, and M. van der Hart, *Vox Sanguinis*, 1973, **24**, 535.

The nature of the modified form of carbohydrate moieties underlying the inherited group-A variant present in the Finnish population (A_{finn}) will be of interest; this is present on the red cells, but apparently not in the saliva.[188] The very low level of A activity on the red cells of an A_m phenotype was accompanied by A, H, and Le^a substances in the saliva, but the A : H ratio was lower than normal.[189]

Collagens

The sequences in collagens of amino-acids neighbouring the hydroxylysine residues that undergo glycosylation are discussed earlier.

Glycosylated hydroxylysine residues, at least in collagen from bone,[190, 191] skin, and tail tendon,[192, 193] are involved in the cross-linking reactions with other lysine and/or hydroxylysine moieties, the reaction needed for the insolubilization and the maturation of collagen. However, not all the cross-linked derivatives are glycosylated in these tissues, nor are they wholly so in granulation tissues and skin.[194] Neither glycosylation nor hydroxylation of lysine residues occurred extensively in the skin collagen (which did not mature) of two patients with an inherited disease resembling, in some ways, the Ehlers–Danlos syndrome.[195]

It has been suggested that the cross-linked entity syndesine exists in a cyclic, hydrogen-bonded form, be it glycosylated or not. A structure of this type is consistent with its unusual stability.[196]

It has been suggested that there are several sites of attachment of carbohydrate in the α_1- and α_2-chains of collagens from the insoluble corium and dentin of cattle. Moreover, it is reported that hexose residues in dentin might be phosphorylated.[197] Rat-skin collagen also has two sites of glycosylation in its α_2-chains.[11] The functions of ε-*N*-glycosyl-lysine and -hydroxylysine residues in collagens are not clear.[198]

A review of the collagen-like proteins of basement membranes has also stressed the importance of at least two non-collagenous glycoproteins in these membranes.[199] Increased urinary excretion of a basement-membrane-like glycoprotein occurred in acute uranium nephropathy.[200] Glycoproteins have been prepared from the glomerular basement membrane of

[188] J. F. Mohn, R. K. Cunningham, A. Pirkola, U. Furnhjelm, and H. R. Nevanlinna, *Vox Sanguinis*, 1973, **25**, 193.
[189] J. Darnborough, D. Voak, and R. M. Pepper, *Vox Sanguinis*, 1973, **24**, 216.
[190] D. R. Eyre and M. J. Glimcher, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 663.
[191] D. R. Eyre and M. J. Glimcher, *Biochem. J.*, 1973, **135**, 393.
[192] S. P. Robins and A. J. Bailey, *F.E.B.S. Letters*, 1974, **38**, 334.
[193] A. J. Bailey and S. P. Robins, *Frontiers in Matrix Biology*, 1973, **1**, 130.
[194] A. J. Bailey, S. Bazin, and A. Delaunay, *Biochim. Biophys. Acta*, 1973, **328**, 383.
[195] R. Pinnell, S. M. Krane, J. E. Kenzora, and M. J. Glimcher, *New England J. Med.*, 1972, **286**, 1013.
[196] N. R. Davis, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 914.
[197] D. Volpin and A. Veis, *Biochemistry*, 1973, **12**, 1452.
[198] M. L. Tanzer, *Science*, 1973, **180**, 561.
[199] N. A. Kefalides, *Internat. Rev. Connective Tissue Res.*, 1973, **6**, 63.
[200] W. R. Griswold and R. M. McIntosh, *Experientia*, 1973, **29**, 575.

normal human kidneys by affinity chromatography of extracts of the membrane; columns of Sepharose, substituted by IgG raised in rabbits against human basement membrane, were employed. Glycoprotein fractions with collagen-type disaccharide units, as well as heterosaccharides, were obtained.[201] Glycopeptides typical of those found in collagen and in non-collagenous glycoproteins have been obtained from collagenous digests of human kidneys.[202]

The interactions between collagen and glycosaminoglycans have been studied by light-scattering measurements; the compositions of the complexes were determined by the type of glycosaminoglycan and by its molecular weight.[203]

Glycosaminoglycans, apart from keratan sulphate, have been found to accelerate the nucleation process involved in the formation of fibres of monomeric tropocollagen prepared from lathyritic rats, although the rate of growth of the fibres is retarded by these substances.[204] These results have added support to the view that fibrillogenesis may be regulated *in vivo* by glycosaminoglycans. An interdependent regulatory mechanism may also exist for catabolism of the components of connective tissue, since adenosine 3′,5′-cyclic phosphate stimulated the activities of both hyaluronidase and collagenase.[205] Another regulatory mechanism may involve inhibition of collagenase by the serum glycoprotein α_2-macroglobulin. A complex of these macromolecules was found in human rheumatoid synovial fluid.[206]

Measurements have been made of the amounts of D-Glc-D-Gal-Hyl and D-Gal-Hyl in urine by an automated procedure,[207] and the method has also been applied to alkaline hydrolysates of basement membranes. It has advantages over earlier procedures[208] used for the determination of hydroxylysine and its derivatives insofar as it can be applied directly to protein hydrolysates.

Evidence has been adduced for the covalent bonding of collagen to the proteoglycans of human costal cartilage. Proteoglycan, which can be extracted from the cartilage only after digestion with collagenase, has been fractionated by density-gradient centrifugation. High-density fractions, in particular, still contained hydroxyproline.[209]

Glycosamino-acids, D-Gal-Hyl and D-Glc-D-Gal-Hyl, were among the products obtained when extracts of the corneal stroma of the squid *Sepia officinalis* were digested sequentially with collagenase and pronase.[210]

[201] P. M. Mahieu and R. J. Winand, *European J. Biochem.*, 1973, **37**, 157.
[202] P. Bardos, M. Lanson, P. Degand, and J. P. Muh, *Clinica Chim. Acta*, 1973, **48**, 27.
[203] B. Öbrink and L.-O. Sundelöf, *European J. Biochem.*, 1973, **37**, 226.
[204] B. Öbrink, *European J. Biochem.*, 1973, **34**, 129.
[205] E. Harper and B. P. Toole, *J. Biol. Chem.*, 1973, **248**, 2625.
[206] S. Abe and Y. Nagai, *J. Biochem. (Japan)*, 1973, **73**, 897.
[207] R. Askenasi, *Biochim. Biophys. Acta*, 1973, **304**, 375.
[208] R. S. Askenasi and N. A. Kefalides, *Analyt. Biochem.*, 1972, **47**, 67.
[209] T. K. Tobayashi and V. Pedrini, *Biochim. Biophys. Acta*, 1973, **303**, 148.
[210] M. Moczar and E. Moczar, *Comp. Biochem. Physiol.*, 1973, **45B**, 213.

In contrast to vertebrate stroma, 97% of the dry weight of the stroma was extractable into molar calcium chloride solution.

The carbohydrate distribution in the collagen of the jelly-fish *Aurelia coerulea* remains to be elucidated.[211]

Glycogens

Several glycogens have been purified by chromatography on agarose gels having a low degree of cross-linking,[212] and the procedure is likely to be useful in examining glycogens obtained from patients with inborn errors of glycogen metabolism.[213] There is platelet dysfunction in type I glycogen-storage (von Gierke's) disease.[214] The preparation of a paracrystalline form of rabbit-liver glycogen, which gives a B-type *X*-ray diffraction pattern,[215] leads to the hope of a better understanding of the stereochemistry of this storage polysaccharide (or glycoprotein, see p. 294).

The effect of treatment of glycogen with alkali upon its chromatographic behaviour has been assessed.[216]

Although mature human erythrocytes have no significant glycogen store, active metabolism of glycogen does occur.[217] An accumulation of glycogen has been found in the rhombencephalon of chicken embryos during development.[218]

A glycogen-metabolizing organelle has been separated from rat liver as a complex with concanavalin A. The glycogen-associated proteins were solubilized by activating phosphorylase so that glycogen was digested.[219] Controls involved in the metabolism of glycogen have been discussed.[220] There was a reduction in the hexokinase (E.C. 2.7.1.1) activity in the soluble fraction of hearts from both starved and streptozotocin diabetic rats, but there was little or no change in the glycogen synthetase and phosphorylase activities. However, levels of glycogen synthetase phosphatase are lower in the diabetic heart, and it is proposed that this underlies the decreased synthesis of glycogen in diabetics.[221] Treatment with insulin restored the level of the phosphatase and increased the level of the D-glucose 6-phosphate-independent form of glycogen synthetase (synthetase I) in heart. It was found, on the other hand, that insulin caused a decrease in synthetase I kinase activity in perfused rat liver, resulting in a higher level of synthetase I activity.[222] These two effects would have the same net result. The rate of conversion of synthetase D (the D-glucose 6-phosphate-dependent form)

[211] B. J. Rigby and M. Hafey, *Austral. J. Biol. Sci.*, 1972, **25**, 1361.
[212] J. J. Marshall, *J. Chromatog.*, 1973, **77**, 201.
[213] F. Huijing, *Ann. New York Acad. Sci.*, 1973, **210**, 290.
[214] E. E. Czapek, D. Deykin, and E. W. Salzman, *Blood*, 1973, **41**, 235.
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[216] L. N. Bobrova and B. I. Stepanenko, *Doklady Akad. Nauk S.S.S.R.*, 1973, **209**, 980.
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[221] I. Des, *Canad. J. Biochem.*, 1973, **51**, 637.
[222] T. B. Miller and J. Larner, *J. Biol. Chem.*, 1973, **248**, 3483.

into synthetase I mediated by insulin was reduced in the presence of potassium ions, but increased by lithium ions. It was concluded that the effect of insulin on glycogen synthetase is related to the Na^+/K^+-activated ATPase.[223] The I-forms of glycogen synthetase in liver, kidney, brain, heart, and skeletal muscle of frogs and rabbits contain serine residues in the requisite sequences for phosphorylation.[224] These are all closely similar to, and possibly identical with, the sequence -Ile-Gln-SER-Val-Arg-, where SER represents the potential phosphate acceptor (*i.e.* the sequence identified earlier for the enzyme from rabbit skeletal muscle).

Conversion of the D-form of glycogen synthetase of rat kidney into the I-form is inhibited by glycogen, fluoride, and ATP; this is also true of the enzyme from other sources. Total synthetase activity is greatest in the outer cortex and cortico-medullary junction and least in the inner medulla.[225] The D (phosphorylated)-form of the enzyme was shown to be strongly inactivated by several disulphides, including cystine, cystamine, homocystine, and oxidized glutathione.[226] The I-form of the enzyme was strongly activated by cellular tricarboxylic acids (citrate, isocitrate, and succinate); this may be important physiologically.[227] Pig-kidney glycogen synthetase (I or D) has been purified to apparent homogeneity; it consists of four subunits (each of molecular weight 9.2×10^4) and contains a large excess of acidic over basic amino-acid residues.[228] The K_m value for the D-enzyme in adipose tissue was lowered in the presence of D-glucose 6-phosphate, but the latter substance had no effect on the Michaelis–Menten constants for the I-form.[229]

A reduced level of glycogen synthetase was found in mouse liver in endotoxaemia, and this is probably responsible for depletion of the carbohydrate stores occurring in this case.[230]

Glycogen synthetase D has been prepared from human polymorphonuclear leucocytes by a technique involving chromatography on a concanavalin A–Sepharose column, and I-enzyme may be easily made from it in a high degree of purity.[231] This method is likely to be of great value in studies of the latter enzyme.

Glycogen synthetase *b* (*i.e.* the D-form [232]) from rat liver has been shown to have a molecular weight of 2.6×10^5 and to undergo further aggregation. The molecular weight of the subunit is *ca.* 8.5×10^4.[233]

It has been suggested that glycogen synthetase-D phosphatase (purified from rabbit skeletal muscle) is capable of dephosphorylating histone

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phosphate.[234] As with the enzyme from rat heart, there are probably complicated controls of the rate of reaction effected by ions and glycogen.[235]

Synthesis of glycogen is probably the regulatory step in normal control of the levels of glycogen in neonatal rat liver.[236] There is a very rapid decrease in the amount of glycogen in the liver of rabbits following death and, in the initial phases, loss is predominantly of the forms of highest molecular weight.[237]

It has been suggested that there is a feed-back regulation of the conversion of phosphorylase-*b* into -*a*, and that this may be indirectly mediated by the level of free, intracellular calcium ions, although the nature of the putative effector(s) is unknown.[238] Glycogen phosphorylase, as well as acid and neutral α-1,4-glucosidases, has been found in homogenates of circulating lymphocytes from normal subjects.[239] The action pattern of the phosphorylase–glycogen complex from rabbit skeletal muscle has been investigated.[240]

Administration of D-fructose to patients with an hereditary fructose intolerance rendered them unresponsive to the hyperglycaemic action of glucagon. Inhibition of phosphorylase *a* by D-fructose 1-phosphate may explain the effect observed *in vivo*.[241] The activation of glycogen phosphorylase induced by epinephrine was inhibited by *Bordetella pertussis* vaccine, but sensitivity to the hormone could be restored by prednisolone.[242] The reasons for the effects are unknown.

The glycogen molecule appears to contain a relatively large number of clusters of α-limit dextrin centres; shellfish glycogen has 23 units per molecular weight of 10^6 and these units are probably randomly dispersed, rather than being in a core.[243]

A marked increase was observed in the glycogen levels of the levator ani muscle of castrated rats following subcutaneous injection with testosterone. The levels of soluble hexokinase and glycogen synthetase I increased, and so too did the level of total glycogen phosphorylase.[244] The effects of insulin and testosterone on the glycogen-synthetase activity in rat perineal muscle have also been studied.[245] Changes in the levels of glycogen in skeletal muscles of rats given an antiandrogen (cyproterone acetate) have been determined.[246]

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The glycogen in leg muscle extractable with cold trichloroacetic acid and the residual glycogen extractable with hot 20% potassium hydroxide are stores that are used, largely sequentially, during exercise by the dytiscid beetle *Cybister confusus*.[247]

Incubation of rat skeletal muscle with methadone led to depressed levels of both glycogen synthetase and phosphorylase; effects not necessarily related to its pharmacological behaviour.[248]

Isolated rat parenchymal cells metabolized carbohydrates, including glycogen, in a manner similar to that of intact perfused liver.[249] They were stimulated by D-glucose to produce glycogen,[250] the level of which increased when insulin was added.[251] Glycogen also accumulated in cultured skin fibroblasts provided that an adequate supply of D-glucose was present.[252]

A simplified procedure for determining the levels of α-glucosidase in the urine of patients is likely to be useful in the detection of Pompe's disease.[253]

Glycosaminoglycuronans, Glycosaminoglycans, and their Protein and Peptide Derivatives

The chemistry of the glycosaminoglycans,[254] including heparin,[255] has been discussed in relation to their activities. This relationship will be better understood when details of the stereochemistry become known.[256] Chondroitin sulphates and other glycosaminoglycans in a number of tissues contain silicon in a bound form as silanolate; it is thought silicon may play a role in the architecture of connective tissues.[257] The core protein of keratan sulphate from bovine nasal cartilage is believed to have a molecular weight *ca.* 2.0×10^5, and to consist of up to sixty chains of keratan sulphate of variable lengths, with a weight average of 8.5×10^3, built on to the protein core *via* alkali-labile linkages.[258] Chondroitin sulphates from ox nasal septum and lamprey cartilage, as well as keratan sulphate from human costal cartilage, exhibit a wide molecular-size disparity, as shown by polyacrylamide-gel electrophoresis.[259] Chains of chondroitin sulphate in the proteoglycan, isolated by extraction of bovine nasal septum with lithium bromide, were shown to have an average molecular weight of *ca.* 2.1×10^4, in reasonable agreement with earlier results; that from

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[252] B. D. Lake and J. C. Marsden, *Biochem. J.*, 1972, **130**, 5P.
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[254] J. F. Kennedy, *Biochem. Soc. Trans.*, 1973, **1**, 807.
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[259] D. Hsu, P. Hoffman, and T. A. Mashburn, *Analyt. Biochem.*, 1973, **52**, 382.

invertebral disc, mainly chondroitin 6-sulphate, was shown to have a molecular weight of *ca.* 1.35×10^4.[260] Since the proteoglycans of bovine nasal septum have a weight-average molecular weight of *ca.* 2.3×10^6,[261] there may be in the order of a hundred chains of chondroitin sulphate linked to a central protein core. However, strict interpretation of the results should be avoided because of the aggregation of proteoglycans, which is referred to later. There is heterogeneity in the size of the chondroitin sulphate and keratan sulphate of guinea-pig cartilage and nucleus pulposus. This is paralleled by a metabolic heterogeneity (apart from the keratan sulphate of nucleus pulposus), as there are fast- and slow-metabolizing pools or fractions.[262]

It has been postulated that intercellular glycosaminoglycans normally restrain the proliferation of cells and that a dynamic equilibrium is maintained by systems composed of hyaluronidase and its physiological inhibitor. The latter is probably an oligoglycosaminoglycan requiring vitamin C for its synthesis. The implications of these observations have been discussed.[263] Homeostasis in extracellular tissues has been reviewed in relation to glycosaminoglycans and glycoproteins, especially collagen.[264]

Analytical Methods.—Hydrolysis of sulphated proteoglycans by acid can lead to the appearance of artifactual *O*-sulphated derivatives of hydroxy-amino-acids, as well as sugars, among the products. Side-reactions of this type can be minimized by the rapid evaporation of hydrolysates at fairly high temperatures.[265]

A simple, quantitative micro-method has been devised for studying the interaction of the cationic dye Alcian Blue 8GX with acid glycosaminoglycans. The amount of complex present after dissociation is assessed by measuring the quantity of dye spectrophotometrically; quantities of the order of 0.5—10 μg glycosaminoglycan can be measured.[266] The procedure has been applied to urine samples from normals and from Hurler and Sanfilippo subjects.[267] Another procedure for determining total amounts of glycosaminoglycans involves treating them with zirconyl ion to produce insoluble complexes; u.v. absorption measurements are made of a competing reaction of Alizarin Red S with zirconyl ions.[268] However, this method is much less sensitive than that using Alcian Blue 8GX.[266] Other procedures involving electrophoretic separation of glycosaminoglycans, in tests for revealing mucopolysaccharidoses, are discussed later.

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[266] P. D. Whiteman, *Biochem. J.*, 1973, **131**, 343.
[267] P. D. Whiteman, *Biochem. J.*, 1973, **131**, 351.
[268] A. H. Wardi and G. A. Michos, *Analyt. Biochem.*, 1973, **51**, 274.

A more sensitive carbazole reaction has been developed and applied to determinations of glycosaminoglycans in sera;[269] it requires about 10% of the amount of serum used in earlier carbazole procedures.

Stereochemistry.—^{1}H N.m.r. spectroscopic analysis of the acetamido- and *N*-methyl groups of mucopolysaccharides has revealed that hexosamine moieties in these substances are in the $^4C_1$ conformation, whereas sialic acid residues, when present, are in the $^1C_4$ form.[270]

Hyaluronic acid in solution has a complex stereochemistry. Analyses of chiroptical properties have indicated a degree of preferred order, and showed that an increase in concentration and decreases in temperature and in ionization of the carboxy-groups all promote the formation of ordered, cross-linked regions.[271, 272] An interpretation may be made in terms of dissociation of a double-stranded helix into a random-coil conformation. Recent *X*-ray diffraction analysis has indicated that crystalline hyaluronic acid may exist in a molecular conformation of an antiparallel, double-stranded helix with four saccharide units per turn,[273] although a range of conformations is possible depending on the relative humidity, temperature, and tension applied.[274] In a review on the conformations of polysaccharides, it has been suggested that the single-helical form is unlikely to occur extensively in solution because it cannot be stabilized by large co-operative effects.[275]

The helical conformations of acid glycosaminoglycans in solution have been studied by chiroptical measurements of their complexes with Methylene Blue.[276]

The existence of more than one stereochemical molecular form of glycosaminoglycans in the crystalline state is probable, and it is likely that interactions with other molecules affect the overall conformation. Chondroitin 6-sulphate in stretched films can occur in two ordered structures, both having single-stranded helices, but with different turn angles between successive disaccharide units. It is not known whether they are right- or left-handed helices or of three- or eight-fold form.[277] Other workers have also described the three-fold helical type of structure.[278]

Chondroitin 4-sulphate has been shown[279] by *X*-ray diffraction to exist as a three-fold helix in one crystalline form, which is closely similar to one

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[275] D. A. Rees, in 'Carbohydrates', ed. G. O. Aspinall, M.T.P. International Review of Science, Series 1, Vol. 7, Butterworths, London, 1974, p. 251.
[276] A. L. Stone, *Biopolymers*, 1972, **11**, 2625.
[277] S. Arnott, J. M. Guss, D. W. L. Hukins, and M. B. Mathews, *Science*, 1973, **180**, 743.
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form of chondroitin 6-sulphate and to hyaluronate.[278, 280] Dermatan sulphate can occur either with an eight-fold helix,[279, 280] like chondroitin 6-sulphate,[35] or with a three-fold helix,[279] like chondroitin 4-[278] and 6-sulphates,[277, 278] in addition to a form with a two-fold helix.[279] The α-L-iduronosyl residues assume the $^4C_1$ conformation in the three-fold helical form of dermatan sulphate,[278, 280] but there is a possibility that these residues occur in the $^1C_4$ form in the eight-fold helix.[280]

Heparan sulphate contains 2-amino-2-deoxy-α-D-glucosyl, α-L-iduronosyl, and, possibly, β-D-glucuronosyl residues. *X*-Ray diffraction analysis revealed little sulphate, and heparan sulphate has been suggested to be composed predominantly of sections of alternating 2-amino-2-deoxy-D-glucose and D-glucuronic acid residues in a repeating structure of the form *O*-β-D-glucuronosyl-(1 → 4)-2-amino-2-deoxy-α-D-glucose.[279]

Composition.—Heparins and heparans from a number of sources, and prepared by different methods, have been shown to have quite different relative amounts of L-iduronic and D-glucuronic acids. Thus, heparan sulphate from beef lung contains about 1.5 times as much L-iduronic acid as D-glucuronic acid, whereas in a preparation from pig mucosa the ratio is about 1.25.[281] The mactins also contain both L-iduronic and D-glucuronic acids,[282] and a preparation from clam tissues has equal proportions of the two acids.[281]

Heparin fractions from the same tissue source have been shown to differ markedly in the content of 2-acetamido-2-deoxy-D-glucose. In addition, preparations from pig mucosa and beef lung were found to contain 27% and 19%, respectively, of this sugar. Heparins with high contents of 2-acetamido-2-deoxy-D-glucose are probably more widespread than previously realized,[283] and heparin from pig intestinal mucosa has been reported to contain arabinose.[284]

The extraction of bovine nasal cartilage by solutions of inorganic salts has been re-examined. Chlorides of the lanthanide metals extracted high yields at much lower concentrations than were achieved with uni- or bi-valent metal ions. It was demonstrated that the molar concentration of the salt at which maximum extraction of proteoglycan occurs is directly proportional to the enthalpy of hydration of the metal ion.[285] Small differences were apparent in analyses of the components isolated under different conditions of salt extraction, although the electrophoretic mobilities of subunits of the substances were practically identical.[286]

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[286] R. W. Mayes, R. M. Mason, and D. C. Griffin, *Biochem. J.*, 1973, **131**, 541.

Nasal cartilage also contains a glycoprotein with a high content of sulphur-containing amino-acids;[287] although it aggregates strongly, it appears not to play a role in the aggregation of glycosaminoglycans.

Proteoglycans extracted from bovine tracheal cartilage with 4M-guanidine hydrochloride solution, when fractionated in gradients of caesium chloride, yielded several components behaving differently on gel filtration.[288] Two different keratan sulphate-protein fractions could be obtained from the products if the proteoglycans were treated first with testicular hyaluronidase and then with 0.1M-lithium hydroxide solution at 3 °C for 103 hours. Whether or not the protein moieties of the two fragments are originally covalently linked is unknown. The larger fraction appeared to contain larger chains of keratan sulphate, which may contain glutamic acid in the carbohydrate–peptide linkage.[289]

The proteoglycans isolated from cattle articular cartilage by extraction with magnesium chloride and with guanidine hydrochloride solutions have been compared. In both cases, the products yielded a 4*S* protein and a 16*S* proteoglycan in associative solvents. Two large proteoglycan aggregates of different size were also found, one in each case, and these may be related to a protein of 2.7*S* which was present in one case; this protein may be important in the organization of connective tissues.[290]

Hyaluronic acid can be localized in tissues by staining methods before and after digestion with *Streptomyces* hyaluronidase, which yields as products only 4,5-unsaturated tetra- and hexa-saccharides that can be readily removed by histochemical techniques. The procedure was applied to umbilical cord, aorta, and cock's comb.[291, 291a]

The presence of arabinose was reported in hyaluronic acid prepared from human and cattle brains, as well as in that from bovine vitreous humour.[292]

Biosynthesis.—Glycosaminoglycans, with the possible exception of hyaluronic acid, require protein backbones to function as initiators. Further evidence has supported the view that chains of chondroitin 4-sulphate become attached to more than one type of polypeptide.[293] Heterogeneous fractions of chondroitin sulphate proteoglycan have also been isolated from rabbit-ear cartilage after incubation with D-[^{14}C]glucose.[294]

No free heparin was found in bovine-liver capsule, but it is present covalently linked through D-xylose to a protein chain.[295] D-Xylose and

[287] M. Janado, S. Cleland, and J. R. Dunstone, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 91.
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β-D-xylosides may be of considerable value as initiators in studying the control mechanism in the biosynthesis of this proteoglycan.[296, 297]

Feedback inhibition of UDP-D-glucose dehydrogenase from cornea and epiphyseal plate cartilage by UDP-D-xylose probably regulates, in part, the cellular concentration of UDP-D-glucuronic acid.[298] In addition, 2-amino-2-deoxy-D-galactose 1-phosphate and UDP-2-amino-2-deoxy-D-galactose also inhibited the rat-liver enzyme by non-competitive and competitive mechanisms, respectively. The lower levels of UDP-D-glucuronic acid found in the liver after injection of 2-amino-2-deoxy-D-galactose into the whole animal may underlie the development of galactosamine hepatitis.[299] UDP-D-Glucose dehydrogenase from *E. coli* was also inhibited competitively by UDP-D-xylose.[300]

The interconversions of the UDP-derivatives of D-glucuronate, L-iduronate, D-xylose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and D-galactose have been reviewed.[301] The levels of intermediates involved in the biosynthesis of glycosaminoglycans in the livers of normal and alloxan diabetic rats have been compared; levels of 2-acetamido-2-deoxy-D-glucose 6-phosphate and ATP are lower in the diabetic animal, whereas those of UDP-2-acetamido-2-deoxyhexose, ADP, AMP, NAD, and NADP are higher.[302] Somantin, sometimes present in the blood of diabetics, stimulated the incorporation of both D-[^{3}H]glucose and [^{35}S]sulphate into the glycosaminoglycans of aorta *in vitro*.[303]

The presence in the microsomal fraction of a mouse mastocytoma of distinct 2-acetamido-2-deoxy-D-glucosyl- and D-glucuronosyl-transferases, which function as catalysts in the production of heparin, has been demonstrated.[304] Exogenous acceptors composed of oligosaccharides linked to serine were prepared from heparin. The first of these enzymes failed to transfer 2-acetamido-2-deoxy-D-glucose to non-reducing, terminal L-iduronic acid residues, but transfer to D-glucuronic acid residues occurred. Similar enzymes have been partially purified from subcellular fractions of pig aorta.[305] Membrane lipids appear to play an important role in the specificity of UDP-D-glucuronyltransferase of guinea-pig liver microsomes; activity of the enzyme *in vitro* was increased by removing phospholipids, but the treated enzyme then developed an ability, absent in the presence of the lipids, to be inhibited by various UDP-sugars.[306] This finding may have wide ramifications.

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Hen's uterus contains a sulphotransferase that catalyses the transfer of sulphate from 3′-phosphono-5′-adenylyl hydrogen sulphate (PAPS) to *N*-desulphated heparan sulphate (the best acceptor), heparan sulphate, *N*-desulphated heparin, and dermatan sulphate; heparin, chondroitin sulphate, and hyaluronic acid did not act as acceptors.[307] Another heparan sulphate sulphotransferase has been partially purified from ox lung; desulphated heparin and desulphated heparan were found to be the best acceptors of sulphate.[308] Endogenous acceptors of sulphate present in microsomes of chicken-embryo epiphyseal cartilage include both chondroitin 4- and -6-sulphates. When incubation of the tissue was carried out at pH 7.8 in the presence of PAPS, only the latter glycosaminoglycan acted as an acceptor, whereas sulphation of both occurred at pH 6.5.[309] The primer for sulphation, and also for the addition of further D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose residues, has a molecular weight estimated to be less than 8×10^3.[310] It seems likely that the sulphating enzymes and the sugar transferases are located in a complex, so that sulphation of the glycosaminoglycan chains is concerted with polymerization of the carbohydrate moieties.[311] Hyaluronic acid was found to inhibit the incorporation of sulphate into material precipitable by cetyl pyridinium chloride in isolated adult chondrocytes.[312]

There was extensive incorporation of [^{35}S]sulphate into the chondroitin sulphate of cultured leucocytes from patients with acute granulocytic leukaemia, but none was incorporated into the cells of patients with acute lymphocytic leukaemia. The finding may possibly be of use in differentiating the two pathological conditions.[313]

The predominant sulphated glycosaminoglycan produced by most lines of cultured, neoplastic, mouse mast cells was shown to be chondroitin sulphate,[314] in agreement with earlier reports. In one line, the product was identified as chondroitin 4-sulphate, and the synthetic activity of these cells was shown to reside in the microsomal fraction.[315]

Production of the predominant proteoglycan fraction produced by cultured chicken-cartilage chondrocytes was reduced in the presence of 5-bromodeoxyuridine. The fraction was completely absent in chondrocytes derived from embryos homozygous for the recessive gene nanomelia.[316] Virus (SV 40 or Herpes simplex type-2)-transformed Syrian hamster-embryo fibroblasts have been shown to produce more hyaluronic acid than do their untransformed counterparts.[317]

[307] A. H. Johnson and J. R. Baker, *Biochim. Biophys. Acta*, 1973, **320**, 341.
[308] T. Foley and J. R. Baker, *Biochem. J.*, 1973, **135**, 187.
[309] M. E. Richmond, S. De Luca, and J. E. Silbert, *Biochemistry*, 1973, **12**, 3898.
[310] M. E. Richmond, S. De Luca, and J. E. Silbert, *Biochemistry*, 1973, **11**, 3904.
[311] S. De Luca, M. E. Richmond, and J. E. Silbert, *Biochemistry*, 1973, **11**, 3911.
[312] O. W. Wiebkin and H. Muir, *F.E.B.S. Letters*, 1973, **37**, 42.
[313] P. Lau, A. J. Gottlieb, and W. J. Williams, *Blood*, 1972, **40**, 725.
[314] R. G. Lewis, A. F. Spencer, and J. E. Silbert, *Biochem. J.*, 1973, **134**, 455.
[315] R. G. Lewis, A. F. Spencer, and J. E. Silbert, *Biochem. J.*, 1973, **134**, 465.
[316] M. J. Palmoski and P. F. Goetinck, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3385.
[317] C. Satoh, R. Duff, F. Rapp, and E. A. Davidson, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 54.

Degradation.—Turnover rates of sulphated heteroglycans have been determined with the aid of [^{35}S]sulphate. Marked differences have been noted in the rates of turnover of both epithelial glycoproteins and connective-tissue glycosaminoglycans, but a full interpretation of these findings is not easy.[318]

Injected chondroitin 4-sulphate (molecular weight 1.8×10^4) may be excreted, in part, in the urine in an undegraded form,[319, 320] although most of the glycosaminoglycan is degraded by the liver.[320] Injected chondroitin 4-sulphate proteoglycan was split into the free polysaccharide before excretion.[319] Cathepsins B1 and D have been suggested to play a part in the catabolism of the cartilage matrix by degrading proteoglycans,[321] and they may be involved in the catabolic route just mentioned;[319] cathepsin D degraded the proteoglycan subunit most rapidly at pH 5. Cathepsin D has been shown to be present in two to three times the normal amount in ulcerated articular cartilage from patients with primary osteoarthritis.[322]

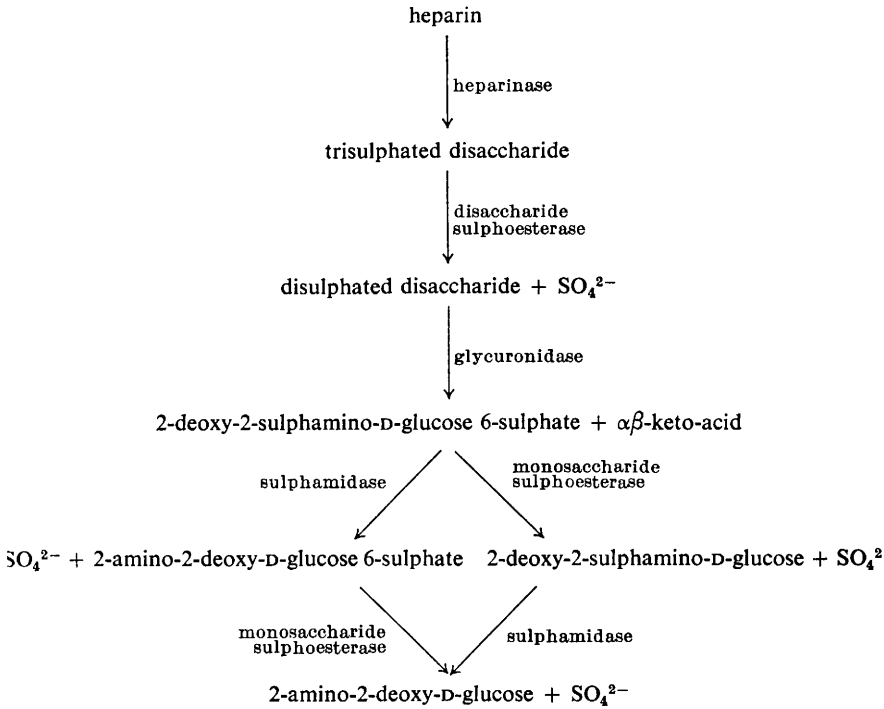
A protease present in the granule fraction of human leucocytes was capable of degrading the non-collagenous glycosaminoglycan matrix at neutral pH in physiological salt solution. Cells overlying cartilage to which aggregated IgG is bound released the enzyme responsible for degradation of the matrix.[323]

Five enzymes capable of degrading heparin to its basic constituents have been purified from *Flavobacterium heparinum*. Degradation of heparin by extracts of these bacteria probably occurs largely by the pathway outlined in Scheme 1.[323a]

Heparin from rat skin was depolymerized into a number of products, separable by gel filtration, on incubation with a 10^5 *g* supernatant from a sonicated homogenate of rat small intestine. Products ranged from inhibitors to activators of lipoprotein lipase, for high- to low-molecular-weight products, respectively.[324]

Normal cultured fibroblasts possess an enzyme capable of releasing the ^{14}C label from a substrate prepared by incubating UDP-D-[^{14}C]Gal*N*Ac with a hexasaccharide from chondroitin 4-sulphate and extracts of chick tibia. Fibroblasts from Tay–Sachs and Sandhoff–Jatzkewitz patients were shown to lack this enzyme, *viz.* β-acetamidodeoxyhexosidase A.[325] If this enzyme normally plays a role in the metabolism of glycosaminoglycans,

[318] T. T. Terho and K. Hartiala, *Biochim. Biophys. Acta*, 1973, **304**, 591.
[319] P. A. Revell and H. Muir, *Biochem. J.*, 1972, **130**, 597.
[320] K. M. Wood, F. S. Wusteman, and C. G. Curtis, *Biochem. J.*, 1973, **134**, 1009.
[321] R. I. G. Morrison, A. J. Barrett, J. T. Dingle, and D. Prior, *Biochim. Biophys. Acta*, 1973, **302**, 411.
[322] A. I. Sapolsky, R. D. Altman, J. F. Woessner, and D. S. Howell, *J. Clin. Invest.*, 1973, **52**, 624.
[323] A. Oronsky, L. Ignarro, and R. Perper, *J. Exp. Med.*, 1973, **138**, 461.
[323a] C. P. Dietrich, M. E. Silva, and Y. M. Michelacci, *J. Biol. Chem.*, 1973, **248**, 6408.
[324] A. A. Horner, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3469.
[325] J. N. Thompson, A. C. Stoolmiller, R. Matalon, and A. Dorfman, *Science*, 1973, **181**, 866.



Scheme 1

it is difficult to understand why there is not extensive accumulation of mucopolysaccharides in the diseases mentioned.

Hyaluronidase from bull-sperm acrosomes appears to be closely similar to, or possibly identical with, the testicular enzyme, but it differs from the lysosomal enzyme present in other tissues.[326]

Ageing.—The role of proteoglycans and collagen of articular cartilage in ageing and in osteoarthritis has been discussed.[327] Levels of the glycosaminoglycans in tissues, in general, change with age and so do those of the hydrolases acting on them.[328] Levels of collagen and bound 2-amino-2-deoxy-D-glucose (probably as keratan sulphate) of the knee-joint and ear cartilages of cattle increase with age, whereas the levels of chondroitin sulphate fall.[329] Less proteochondroitin sulphate can be extracted with phosphate buffer (pH 7.4 and p*I* 0.14) from these sources as the animal ages.[330]

[326] L. J. D. Zaneveld, K. L. Polakoski, and G. F. B. Schumacher, *J. Biol. Chem.*, 1973, **248**, 564.
[327] C. A. McDevitt, *Ann. Rheumatic Diseases*, 1973, **32**, 364.
[328] 'Connective Tissue and Ageing', ed. H. G. Vogel, Excerpta Medica, Amsterdam, 1973.
[329] P. L. Muthiah, K. Braeumer, and K. Kuehn, *Carbohydrate Res.*, 1973, **30**, 211.
[330] P. L. Muthiah and K. Kuehn, *Biochim. Biophys. Acta*, 1973, **304**, 12.

Proteoglycans of small hydrodynamic size from pig laryngeal and articular cartilage have been shown to be immunologically identical and to be present in cartilage, in small amounts, before birth, as well as in mature animals.[331] Digestion of the larger proteoglycans with hyaluronidase gave rise to a number of precipitin lines, one of which was identical with that given by a small proteoglycan.

Old people (63—79 years) have been shown to excrete less glycosaminoglycans in urine (1.74 ± 0.51 mg day^{-1} as uronic acid) than do young, healthy males (26—31 years, 4.91 ± 1.23 mg day^{-1}); the nature of these glycosaminoglycans has been examined by two-dimensional electrophoresis on cellulose acetate.[332] Preparative separations of urinary glycosaminoglycans can be carried out in agar gels.[333]

Aggregation and Interaction with Proteins and Peptides.—Two link factors required for the aggregation of cartilage proteoglycan have been isolated by gradient-density centrifugation in caesium chloride.[334] One of them has been identified as hyaluronic acid;[335] a decasaccharide prepared from hyaluronic acid was the smallest fragment able to bind strongly to the proteoglycan.[336] The nature of a third aggregation factor of small molecular size, which is present in rabbit-ear cartilage, was not determined.[337] It has been shown that there is more than one component in the glycoprotein-link fraction originally isolated by Hascall and Sajdera from cattle trachea.[338] Association of proteoglycans may possibly occur in the absence of extraneous factors,[339] but the relationship between the aggregates produced in the presence of specific factors and any formed in their absence is unknown.

Chiroptical methods have shown that poly-L-arginine [110] is bound more strongly than poly-L-lysine [111] to chondroitin 6-sulphate in aqueous solution at neutral pH. Both polypeptides largely adopt α-helical configurations in the complexes formed, rather than charged-coil configurations seen in the absence of either chondroitin 6-sulphate [340, 341] or heparin.[342] Chondroitin 4-sulphate has been found less effective than chondroitin 6-sulphate in converting poly-L-lysine into the α-helical form.[343]

Hyaluronic acid from human synovial fluid has been shown to bind to bovine serum albumin in a non-specific way.[344]

[331] K. D. Brandt, C. P. Tsiganos, and H. Muir, *Biochim. Biophys. Acta*, 1973, **320**, 453.
[332] S. Ohkawa, R. Hata, Y. Nagai, and M. Sugiura, *J. Biochem. (Japan)*, 1972, **72**, 1495.
[333] N. Taniguchi, *Clinica Chim. Acta*, 1972, **42**, 221.
[334] J. D. Gregory, *Biochem. J.*, 1973, **133**, 383.
[335] T. E. Hardingham and H. Muir, *Trans. Biochem. Soc.*, 1973, **1**, 282.
[336] T. E. Hardingham and H. Muir, *Biochem. J.*, 1973, **135**, 905.
[337] B. Rokosova, A. N. Hanson, and J. P. Bentley, *Biochim. Biophys. Acta*, 1973, **320**, 442.
[338] D. Heinegård, *Biochim. Biophys. Acta*, 1972, **285**, 181.
[339] P. J. Wells and A. Serafini-Fracassini, *Nature New Biol.*, 1973, **243**, 266.
[340] R. A. Gelman, D. N. Glaser, and J. Blackwell, *Biopolymers*, 1973, **12**, 1223.
[341] R. A. Gelman, W. B. Rippon, and J. Blackwell, *Biopolymers*, 1973, **12**, 541.
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[343] R. A. Gelman and J. Blackwell, *Biochim. Biophys. Acta*, 1973, **297**, 452.
[344] J. V. Smith, P. Ghosh, and T. K. F. Taylor, *Proc. Austral. Biochem. Soc.*, 1973, **6**, 35.

The semilunar cartilage of the knee joint is composed of a glycosaminoglycan containing dermatan sulphate and chondroitin 6-sulphate as a copolymer. It was suggested that the presence of dermatan sulphate is required in order that the fibrous cartilage may form bundles with collagen.[345]

Activities.—No direct relationship could be demonstrated between the glycosaminoglycan content of human-femoral condylar cartilage and its tensile stiffness and fracture stress.[346]

Studies have been made of the effects of low doses of heparin on the pattern of serum lipids,[347] on the adhesiveness and aggregation of platelets,[348] and on the fibrinolytic activity[349] of normal and atherosclerotic subjects. The known interaction of heparin with gentamicin may lead to errors in assays of the antibiotic by an agar-diffusion method.[350]

Platelet factor 4 (heparin-neutralizing activity) was shown to be released from human-blood platelets by thrombin in the form of a complex with proteoglycan. The latter consists of four chains of chondroitin 4-sulphate (molecular weight 1.2×10^4) linked to a single polypeptide chain. Heparin is capable of displacing the proteoglycan.[351]

Low concentrations of hyaluronic acid inhibited the production of cartilage-like aggregates in high-density, stationary cultures of trypsinized chicken-embyro precartilage cells. Thyroxine, growth hormone, calcitonin, and cyclic AMP prevented this inhibitory effect, leading to the suggestion that hyaluronic acid may have an important role in the regulation of growth.[352]

There is a reduced level of glycosaminoglycans in the sternocostal cartilage of mice with congenital autosomal recessive hydrocephalus; this is associated with a defect in the formation of the sternum. In heterozygotes for the defect, there is an increased level of glycosaminoglycans compared with normals, which is associated with a delay in calcification of the sternum.[353]

More proteoglycan of small molecular size was found in the knee-joint cartilage of lame pigs than in that of normal animals.[354] Concentrations of hyaluronic acid and chondroitin 4-sulphate in the synovial fluid from knee

[345] H. Habuchi, T. Yamagata, H. Iwata, and S. Suzuki, *J. Biol. Chem.*, 1973, **248**, 6019.
[346] G. E. Kempson, H. Muir, C. Pollard, and M. Tuke, *Biochim. Biophys. Acta*, 1973, **297**, 457.
[347] A. Notarbartolo, A. Di Sciacca, and D. Campisi, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 427.
[348] A. Cajozzo, I. Carreca, and V. Abbadessa, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 493.
[349] A. Cajozzo, P. Citarrella, and C. Malleo, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 490.
[350] E. Yourassowsky, M. E. De Broe, and R. J. Wieme, *Clinica Chim. Acta*, 1972, **42**, 189.
[351] A. J. Barber, R. Käser-Glanzmann, M. Jakabova, and E. F. Lüscher, *Biochim. Biophys. Acta*, 1972, **286**, 312.
[352] B. P. Toole, *Science*, 1973, **180**, 302.
[353] M. Breen, R. Richardson, W. Bondareff, and H. G. Weinstein, *Biochim. Biophys. Acta*, 1973, **304**, 828.
[354] Z. Simůnek and H. Muir, *Biochem. J.*, 1972, **130**, 181.

joints of patients with rheumatoid arthritis were about half those in normals.[355] It has been shown that hyaluronic acid makes up more than 80% of the glycosaminoglycans of synovial fluid.[356]

A study of consecutive intima-media layers of cattle aorta thoracica has revealed that the inner layers contain higher amounts of glycosaminoglycans than do the outer layers, and that the distribution of glycosaminoglycans changes.[357]

Levels of Glycosaminoglycans in Tissues.—There is a marked difference in the contents of chondroitin 4- and 6-sulphates between the epiphyseal growth cartilages of vertebrae and long bones (femur and humerus) of man.[358] The effects of denervation and subsequent stimulation of these muscles on the glycosaminoglycan content of the latter have been studied.[359]

In tadpole-back skin, dermatan sulphate was found to be concentrated in the basal lamellar fraction, whereas heparan sulphate, heparin, and chondroitin sulphate were concentrated in the epithelial part.[360]

Oral administration of either tolbutamide or phenformin to rats for a period of two months led to increased levels of sulphated glycosaminoglycans in the aorta, although the level of hyaluronic acid fell.[361] There was also a decreased level of hepatic UDP-D-glucose dehydrogenase.[362]

Heparan sulphate has been isolated from beef lung and aorta, from human amyloid liver and intestine, and from the urine of a Sanfilippo patient; it was shown to consist of a family of related polymers.[363]

Pathology of Mucopolysaccharides.—Revised editions of classical works [364, 365] include a restatement of the six clinically defined mucopolysaccharidoses; however, Hurler (type I) and Scheie (type V) diseases are not presently distinguishable by means of studies with cultured fibroblasts.[365] The enzyme defects and problems arising in several types were also discussed by one of the pioneers in the field of the corrective factors involved.[366] Reduced levels of β-D-galactosidase activity found in the liver of patients with mucopolysaccharidoses are probably due to inhibition of

[355] J. A. Kofoed, A. A. Tocci, and A. C. Barcelo, *Experientia*, 1973, **29**, 680.
[356] P. O. Seppala, J. Karkkainen, A. Lehtenen, and P. Makisara, *Clinica Chim. Acta*, 1972, **36**, 549.
[357] M. A. Hodara, K. von Figura, I. Filipovic, and E. Buddecke, *Z. physiol. Chem.*, 1973, **354**, 445.
[358] P. A. S. Mourao and C. P. Dietrich, *Biochim. Biophys. Acta*, 1973, **320**, 210.
[359] A. A. Novykova and L. H. Klymenko, *Ukrain. biokhim. Zhur.*, 1973, **45**, 398.
[360] R. Hata and Y. Nagai, *Biochim. Biophys. Acta*, 1973, **304**, 408.
[361] K. Prasannan and P. A. Kurup, *Indian J. Biochem. Biophys.*, 1973, **10**, 42.
[362] K. Prasannan and P. A. Kurup, *Indian J. Biochem. Biophys.*, 1973, **10**, 68.
[363] A. Linker and P. Hovingh, *Carbohydrate Res.*, 1973, **29**, 41.
[364] A. Dorfman and R. Matalon, in 'The Metabolic Basis of Inherited Disease', ed. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, 3rd edn., McGraw-Hill, New York, p. 1218.
[365] V. A. McKusick, 'Heritable Disorders of Connective Tissue', 4th edn., C. B. Mosby Co., St. Louis, p. 521.
[366] E. F. Neufeld and M. Cantz, in 'Lysosomes and Storage Diseases', ed. H. G. Hers and F. van Hoof, Academic Press, New York, 1973.

the enzyme by the accumulated glycosaminoglycans,[367] as has been suggested earlier.[368]

The corrective factor (α-L-iduronidase) for the Hurler (type I) autosomal recessive syndrome has been assessed by determining its effect on the rate of loss of sulphated mucopolysaccharides from cultured Hurler fibroblasts. The cells were harvested with trypsin, and the mucopolysaccharide was precipitated with cetyl pyridinium chloride.[369] Hurler fibroblasts were found to synthesize hyaluronic acid more rapidly than do normal cells.[370]

Studies with cultured fibroblasts showed that the Hunter X-linked (type II) syndrome involves a deficiency of sulphoiduronate sulphatase.[371, 372] The abnormal storage of dermatan and heparan sulphates in this disease leads to other abnormalities of enzyme patterns in tissues; notably, increased activities of β-2-acetamido-2-deoxyhexosidase and β-glucuronidase, especially in liver.[373]

Clinical type III autosomal recessive mucopolysaccharidosis is biochemically of two forms: Sanfilippo A disease was shown to be likely to involve a deficiency of heparan sulphate sulphamidase.[374, 375] Sanfilippo B disease, which also involves abnormal storage of heparan sulphate, most probably results from a deficiency of α-2-acetamido-2-deoxyglucosidase.[376–378]

Glycosaminoglycans present in the urine of three patients with the Sanfilippo syndrome have been examined after infusions of plasma.[379] There were two types of response: that of one of the patients differed from the response of the other two, who were brother and sister. The first of these patients may well have Sanfilippo A disease.

The urine of a patient with the autosomal recessive Morquio (type IV) syndrome has been found to contain excesses of a chondroitin sulphate and keratan sulphate.[380]

It has been confirmed that the Scheie autosomal recessive syndrome (type V), like the Hurler syndrome, arises from a deficiency of α-L-iduronidase.[381]

A previously unclassified type of mucopolysaccharidosis has been described [382] in which there is an almost complete absence of the lysosomal

[367] J. A. Kint, *F.E.B.S. Letters*, 1973, **36**, 53.
[368] R. D. Marshall and A. Neuberger, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 407.
[369] J. K. Herd, *Proc. Soc. Exp. Biol. Med.*, 1973, **143**, 446.
[370] R. J. Germinario, A. Kahlenberg, and L. Pinsky, *Biochem. J.*, 1973, **132**, 403.
[371] G. Bach, F. Eisenberg, M. Cantz, and E. F. Neufeld, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 2134.
[372] I. Sjöberg. L.-Å. Fransson, R. Matalon, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1125.
[373] P. A. Roukema, C. H. Oderkerk, and G. van den Berg, *Clinica Chim. Acta*, 1973, **44**, 277.
[374] H. Kresse and E. F. Neufeld, *J. Biol. Chem.*, 1972, **247**, 2164.
[375] R. Matalon and A. Dorfman, *Paediatric Res.*, 1973, **7**, 384.
[376] J. S. O'Brien, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1720.
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[378] J. S. O'Brien, A. L. Miller, A. W. Loverde, and M. L. Veath, *Science*, 1973, **181**, 753.
[379] M. F. Dean, H. Muir, and P. F. Benson, *Nature New Biol.*, 1973, **243**, 143.
[380] D. A. Applegarth, G. Bozoian, and R. B. Lowry, *Biochem. Soc. Trans.*, 1973, **1**, 847.
[381] R. Matalon and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 959.
[382] W. S. Sly, B. A. Quinton, W. C. McAlister, and D. L. Rimoin, *J. Pediat.*, 1973, **82**, 249.

enzyme β-D-glucuronidase in cultured fibroblasts [383] and in lysates of white blood cells.[382] A mucopolysaccharidosis is also now known in which there is an excretion of chondroitin 6-sulphate with a low content of sulphate.[384]

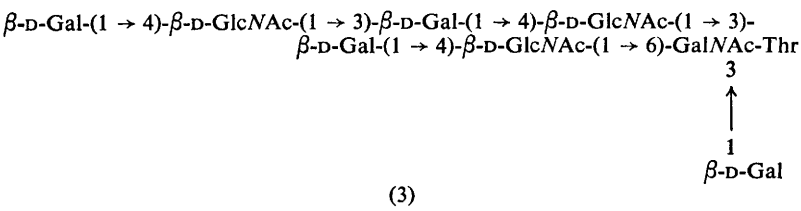
A secondary induced abnormal distribution of lysosomal isoenzymes has been found in mucopolysaccharidoses,[385] including a striking reduction of β-D-galactosidase activity.[367, 386]

In many patients examined, a close correlation has been demonstrated between the types of mucopolysaccharide excreted in the urine and the clinical picture of a specific mucopolysaccharidosis.[387] This procedure, like that used for measurements on amniotic fluid,[388] also identified the glycosaminoglycan by its mobility on electrophoresis. An early, prenatal diagnosis of Hurler's syndrome has been achieved by examination of the glycosaminoglycans in amniotic fluid, and confirmation was obtained by examining the uptake of sulphate by cultured fibroblasts from the abortus.[389] A simple turbidimetric procedure has been developed for screening children's urine for hypermucopolysacchariduria.[390]

The Niemann–Pick disease is characterized by a deficiency of phospholipase C, which specifically cleaves sphingomyelin. Secondary to an increase in the level of sphingomyelin in tissues, there are increases in the levels of glycoproteins and glycosaminoglycans in brain.[391] By contrast, little or no change from normal levels of these substances in brain was evident in Krabbe's disease, in which there is a deficiency of galactocerebroside β-D-galactosidase.[392]

A glycopeptide containing D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and threonine, in the molar ratios 4 : 3 : 1 : 1, has been isolated from papain digests of the liver from a subject who had died from GM_1-gangliosidosis.[393] Structure (3) was tentatively suggested for this glycopeptide, which is believed to form part of a keratan-sulphate-like storage material; the deficiency of β-D-galactosidase in GM_1-gangliosidosis perhaps explains the accumulation of this material.

[383] C. W. Hall, M. Cantz, and E. F. Neufeld, *Arch. Biochem. Biophys.*, 1973, **155**, 32.
[384] P. A. S. Mourao, S. A. P. Toledo, H. B. Nader, and C. P. Dietrich, *Biochem. Med.*, 1973, **7**, 415.
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[389] M. d'A. Crawfurd, M. F. Dean, D. M. Hunt, D. R. Johnson, R. R. MacDonald, H. Muir, E. A. Payling Wright, and C. R. Payling Wright, *J. Med. Genetics*, 1973, **10**, 144.
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[391] E. G. Brunngraber, B. Berra, and V. Zambotti, *Clinica Chim. Acta*, 1973, **48**, 173.
[392] B. Berra, E. G. Brunngraber, V. Aguiler, A. Aro, and V. Zambotti, *Clinica Chim. Acta*, 1973, **47**, 325.
[393] G. C. Tsay and G. Dawson, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 759.



(3)

A substance resembling hyaluronic acid, designated proteohyaluronic acid, has been isolated from the cyst-substance in cystic mucoid degeneration, but no evidence for a covalent linkage between protein and hyaluronic acid was provided.[394] The circulatory disturbance caused by compression of the vascular lumen in this disease might be due to retention of water by this substance.

Mammalian Bone, Cell, and Tissue Glycoproteins

A myelin glycoprotein identified as a major component of sciatic nerves [395] may be identical with the P_0- or J-protein described previously.[396, 397] The effects of denervation on muscle glycoproteins in rabbits were studied.[398]

Both 2-amino-2-deoxy-D-[^{14}C]glucose and plasma glycoprotein containing this labelled sugar have been used in studies on the synthesis of the organic matrix by bone tissue. There are several glycoproteins in bone, one of which is an α-glycoprotein that also appears to be present in sera.[399]

Cattle-liver sulphatase A has been confirmed as a glycoprotein. It contains D-galactose (8), D-mannose (14), 2-amino-2-deoxy-D-glucose (18), and sialic acid (8 residues per unit molecular weight of 1.07×10^5); small amounts of L-fucose and D-glucose, the latter possibly an impurity, were also found.[400] No obvious structural relation between sulphatases A and B is known. The B-enzyme exhibited no activity against cerebroside sulphate and it does not have any obvious chondrosulphatase or sulphotransferase activities.[401, 402]

The secretory vesicles of the Golgi apparatus of rat liver contain a UDP-galactose:2-acetamido-2-deoxyglucose galactosyltransferase that may be of importance in glycosylating very low-density lipoproteins.[403] Enzyme markers and electron microscopy were used for monitoring the subcellular

[394] M. Endo, S. Tamura, S. Minakuchi, H. Ouchi, and Z. Yosizawa, *Clinica Chim. Acta*, 1973, **47**, 417.
[395] J. G. Wood and R. M. C. Dawson, *J. Neurochem.*, 1973, **21**, 717.
[396] S. W. Brostoff and E. H. Eylar, *Arch. Biochem. Biophys.*, 1972, **153**, 590.
[397] J. Csejtey, J. F. Hallpike, C. W. M. Adams, and O. B. Bayliss, *J. Neurochem.*, 1972, **19**, 1931.
[398] R. Cotrufo and S. H. Appel, *Boll. Soc. Ital. Biol. Sper.*, 1973, **49**, 21.
[399] J. F. Triffilt and M. Owen, *Biochem. J.*, 1973, **136**, 125.
[400] E. R. B. Graham and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **329**, 88.
[401] W. S. Blesynski and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **317**, 164.
[402] A. Jerfy and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **293**, 178.
[403] W. D. Merritt and D. J. Morré, *Biochim. Biophys. Acta*, 1973, **304**, 397.

fractions examined. These techniques have been reviewed;[404] their application is essential in studies of the cellular topography of glycoprotein-forming sites. Glycosyltransferases in secretory vesicles may also be involved in the production of plasma-membrane-like vesicles capable of fusing with, and of contributing new membrane to, the plasma membrane.[405]

Glycopeptides isolated from rough and smooth microsomes of rat liver have been shown to differ; those from rough membranes largely contain neutral oligosaccharides, whereas smooth membranes are richer in prosthetic groups containing acidic sugars.[406]

Increased levels of glucosaminyltransferase have been demonstrated in the livers and spleens of animals injected with myxoviruses during the stage of virus multiplication.[407] This is not unlike the changes seen also in chicken-embryo fibroblasts when they are infected with arborvirus.[408] The mannosyltransferases in microsomes and in mitochondria of rat liver are known to have different properties.[409] Considerable differences have been observed in the electrophoretic patterns obtained for proteins extracted (using sodium dodecyl sulphate) from the brush borders of jejunum and ileum. Proteins extracted included a large number of glycoproteins, many of which arose from the membrane.[410]

It has been shown that pig pancreatic amylases I and II both contain, in addition to 470 amino-acid residues each in single polypeptide chains, approximately one residue of 2-amino-2-deoxy-D-glucose and small amounts of D-galactose, D-mannose, and L-fucose. It was suggested that the carbohydrate may not be necessary for enzymic activity.[411]

The asparagine residue carrying a carbohydrate moiety is located in bovine pancreatic deoxyribonuclease A at position 18 of a polypeptide chain 257 residues long. This enzyme appears to carry a further potential site for glycosylation (-Asn-Asp-Ser-) at residues 103—105.[412–414]

A mannosyltransferase, which catalyses the incorporation of D-mannose into an endogenous acceptor, has been found in the membrane fraction of alveolar cells of the lung.[415] The nature of the glycoproteins synthesized by this enzyme is unknown.

[404] D. J. Morré, in 'Molecular Techniques and Approaches in Developmental Biology', ed. M. J. Chrispeels, Wiley–Interscience, New York, 1973, p. 1.
[405] D. J. Morré and H. H. Mollenhauer, in 'Dynamics of Plant Ultrastructure', ed. A. W. Robards, McGraw-Hill, New York, 1974, p. 84.
[406] T. Kawasaki and I. Yamashima, *J. Biochem. (Japan)*, 1973, **74**, 639.
[407] A. Defrene and P. Louisot, *Ann. Inst. Pasteur*, 1972, **122**, 529.
[408] C. Froger and P. Louisot, *Comp. Biochem. Physiol.*, 1972, **43B**, 223.
[409] R. Morelis and P. Louisot, *Compt. rend.*, 1973, **276**, *D*, 2219.
[410] D. Maestracci, J. Schmitz, H. Preiser, and R. K. Crane, *Biochim. Biophys. Acta*, 1973, **323**, 113.
[411] B. Beaupoil-Abadie, M. Raffalli, P. Cozzone, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1973, **297**, 436.
[412] J. Salnikow, T.-H. Liao, S. Moore, and W. H. Stein, *J. Biol. Chem.*, 1973, **248**, 1480.
[413] T.-H. Liao, J. Salnikow, S. Moore, and W. H. Stein, *J. Biol. Chem.*, 1973, **248**, 1489.
[414] S. Moore and W. H. Stein, *Science*, 1973, **180**, 458.
[415] C. Levrat and P. Louisot, *Canad. J. Biochem.*, 1973, **51**, 931.

It has been suggested that cystic fibrosis is primarily caused by an elevated level of various glycosyltransferases in lung microsomes.[416] This hypothesis must be considered in the light of other recent findings that have demonstrated a generalized deficiency of kallikreins in patients with the disease.[417] Further support has been given to the concept of biochemical heterogeneity in the disease, since some cystic-fibrous patients have low levels of serum hexosaminidase, predominantly the B isoenzyme, associated with a low cultured-cell metachromasia, whereas others have a high level of hexosaminidase in the serum. The latter group has mainly the A isoenzyme and their cells exhibit high metachromasia.[418, 419]

A glycopeptide prepared from pig aorta has been shown to possess a terminal structure (4); it also contains D-galactose moieties with branch



(4)

points at positions 4 and 6, as well as D-mannose residues with substituents at positions 2 and 4. Also present are non-reducing, terminal D-galactose, D-mannose, and L-fucose residues.[420] A microsomal mannosyltransferase, presumably related to those involved in the synthesis of such glycoproteins, has been found in aortic intima. It is a very active enzyme requiring Mn^{2+} or Mg^{2+} ions for expression of its activity.[421]

Toad-oviduct glycoprotein has been shown to contain several forms of sialic acid,[422] as do other glycoproteins.[423, 424] Both *N*-acetyl- and *N*-glycolylneuraminic acids, as well as *N*,4-*O*-diacetylneuraminic acid and, probably, its *N*-glycolyl counterpart, are present in toad (*Bufo arenarum*) protein. The glycoprotein is probably a precursor of the jelly secreted by the oviduct during ovulation.[422] Three distinct neuraminidases are present in toad oviduct; somewhat surprisingly, Ba^{2+} and Ca^{2+} ions and various sialic acids activated these enzymes.[425]

The enzyme 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine amidohydralase has been reported to be present in mammalian spermatozoa.[426] Whether this is the origin of the enzyme present in seminal plasma or whether it arises in the spermatozoa by pinocytosis is not clear.

Cyclic AMP has a marked effect on the formation of membrane glycoproteins of cultured cells. It was not possible from studies on Chinese

[416] P. Louisot, C. Levrat, and R. Gilly, *Clinica Chim. Acta*, 1973, **48**, 373.
[417] C. J. S. Rao, L. A. Posner, and H. L. Nadler, *Science*, 1972, **177**, 610.
[418] J. H. Conover, E. Conod, and K. Hirschorn, *Lancet*, 1973, i, 1122.
[419] Editorial Article in *Lancet*, 1973, ii, 307.
[420] B. Kraska and A. Klemer, *Z. physiol. Chem.*, 1973, **354**, 462.
[421] M. Richards, P. Broquet, and P. Louisot, *J. Mol. Cellular Cardiology*, 1972, **4**, 465.
[422] N. R. De Martinez and J. M. Olavarria, *Biochim. Biophys. Acta*, 1973, **320**, 295.
[423] J. A. Cabezas, *Rev. Espan. Fisiol.*, 1973, **29**, 307.
[424] R. Schauer, *Angew. Chem. Internat. Edn.*, 1973, **12**, 127.
[425] N. R. De Martinez and J. M. Olavarria, *Biochim. Biophys. Acta*, 1973, **320**, 301.
[426] V. K. Bhalla, W. L. Tillman, and W. L. Williams, *J. Reprod. Fertility*, 1973, **34**, 137.

hamster-ovary cells to decide whether cyclic AMP alters the proportions of various membrane glycoproteins being produced or whether it leads to changes in the carbohydrate side-chains of some glycoproteins. A number of the changes observed were not unlike the reverse of those accompanying the transformation of fibroblasts by oncogenic viruses.[427] Many transformed cells contained glycoproteins in their membranes possessing increased amounts of sialic acid, a finding in accord with the presence of high levels of sialyltransferase in the cells.[428]

Columns of insolubilized trypsin have been used to release glycoproteins and glycopeptides from Ehrlich ascites tumour cells E_4.[429] The nature and function of these glycoproteins will be of interest.

Hormonal Glycoproteins

Useful reviews have appeared describing many of the biological properties of human chorionic thyrotrophin, chorionic gonadotrophin,[430] and pituitary thyrotrophin,[431] all of which contain covalently bound sugars.

The relationship of a number of the activities of the gonadotrophins to their structures has been discussed,[432] as was also the chemico-biological relationship between the releasing hormones and the follicle-stimulating and luteinizing hormones.[433] The close similarity of the α-chains of pituitary thyrotrophin, pituitary-luteinizing hormone, and chorionic gonadotrophin was mentioned in a review on glycoproteins.[430] Many of the sequences of amino-acids in sheep and human luteinizing hormones are now known.[434] Nitration (with tetranitromethane) of cattle pituitary hormone yielded an inactive product, but the nitrated α-chain recombined with the β-chains of luteinizing hormone and thyrotrophin to give partially active products. Since the tyrosine residues at positions 21, 92, and 93 of the α-chain were nitrated, it was argued that these residues are required for interaction of the hormones with their target receptors.[435]

Emphasis has been placed also on the lipolytic activity in white adipose tissue of luteinizing hormone and its subunits. Stimulation of the production of lactic acid *in vitro* in prepubertal rat ovaries was also studied, although these activities have yet to be examined in the light of the part played by the carbohydrate moieties.[436]

[427] M. M. Baig and R. M. Roberts, *Biochem. J.*, 1973, **134**, 329.
[428] L. Warren, J. P. Fuhrer, and C. A. Buck, *Fed. Proc.*, 1973, **32**, 80.
[429] U. Kocemba-Sliwowska and J. Kawiak, *Acta Biochim. Polon.*, 1973, **20**, 113.
[430] B. N. Saxena, *Vitamins and Hormones*, 1971, **29**, 95.
[431] J. E. Dumont, *Vitamins and Hormones*, 1971, **29**, 287.
[432] J. F. Kennedy, *Endocrinol. Experimentalis*, 1973, **7**, 5.
[433] J. F. Kennedy, L. J. Gray, S. Ramanvongse, L. Albrighton, and W. F. White, *Life Sciences Part I*, 1973, **12**, 533.
[434] D. N. Ward, L. E. Reichert, W.-K. Liu, H. S. Nahm, J. Hsia, W. M. Lamkin, and N. S. Jones, *Recent Progr. Hormone Res.*, 1973, **29**, 533.
[435] K.-W. Cheng and J. G. Pierce, *J. Biol. Chem.*, 1972, **247**, 7163.
[436] H. Papkoff, M. R. Sairam, S. Walker Farmer, and C. H. Li, *Recent Progr. Hormone Res.*, 1973, **29**, 563.

Antibody to human follicle-stimulating hormone has been attached to cellulose 2,3-cyclic carbonate, and the product was used in a solid-phase radioimmunoassay of unknown amounts of the hormone. The assay involved studies of competitive binding using a radiolabelled hormone.[437]

About 75% of the L-fucose residues of human pituitary follicle-stimulating hormone was released by treatment at pH 2.0 at 80 °C for 50 minutes, the mildest conditions suitable for removing sialic acid residues from this glycoprotein.[438]

Some of the approaches used in developing a system for the assay of luteinizing hormone *in vitro* have been described.[439]

The single carbohydrate moieties of the β-chains of pituitary-luteinizing hormones of both pig and cattle are attached at asparagine-13.[440] The two carbohydrate moieties of the pig α-chain have been shown to be situated in analogous positions to those in the sheep α-chain. The total amino-acid sequence of the pig hormone is now known.[440, 441]

An improved purification of human chorionic gonadotrophin has been described.[442] Twice as much sialic acid is present in the α-chain as in the β-subunit.[443] The amino-acid sequence of the α-chain is slightly different from the α-chain of luteinizing hormone, with a 2-residue inversion and a 3-residue deletion at the *N*-terminus. Carbohydrate is attached at asparagine residues 52 and 78 of human chorionic gonadotrophin, where the sequences are -Asn-Val-Thr- and -Asn-His-Thr-, respectively.[444] There are five oligosaccharide units in the β-chain of this hormone, two of them at asparagine residues 13 and 30, where the sequences are -Asn-Ala-Thr- and -Asn-Thr-Thr-, respectively.[445] Serine residues 118, 129, and 131 are also glycoslylated; serine-118 occurs in the middle of the pentapeptide Gln-Asp-Ser-Ser-Ser, and residues 127 to 133 consist of Leu-Pro-Ser-Pro-Ser-Arg-Leu. There is no carbohydrate chain at position 126, contrary to an earlier report,[446] and the assignment of the carbohydrate chain to position 118 is tentative, since it could occur at either position 119 or 120.[445]

Human chorionic gonadotrophin may function as a glycogenolytic agent in placental tissue. On immature placental villi placed in culture,

[437] J. F. Kennedy and H. Cho Tun, *Carbohydrate Res.*, 1973, **30**, 11.
[438] J. F. Kennedy and M. F. Chaplin, *J. Endocrinol.*, 1973, **57**, 501.
[439] L. E. Reichert, F. Leidenberger, and C. G. Trowbridge, *Recent Progr. Hormone Res.*, 1973, **29**, 497.
[440] G. Maghuin-Rogister and G. Hennen, *European J. Biochem.*, 1973, **39**, 235.
[441] G. Maghuin-Rogister, Y. Combarnous, and G. Hennen, *European J. Biochem.*, 1973, **39**, 255.
[442] H. Okumura, S. Namba, and S. Matsushima, *Endocrinol. Japon.*, 1973, **20**, 67.
[443] R. C. Shownkeen, M. B. Thomas, and A. Stockell Hartree, *J. Endocrinol.*, 1973, **59**, 201.
[444] R. Bellisario, R. B. Carlsen, and O. P. Bahl, *J. Biol. Chem.*, 1973, **248**, 6796.
[445] R. B. Carlsen, O. P. Bahl, and N. Swaminathan, *J. Biol. Chem.*, 1973, **248**, 6810.
[446] O. P. Bahl, R. B. Carlsen, R. Bellisario, and N. Swaminathan, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 416.

the hormone had the effect of lowering the level of glycogen and of raising the levels of glycogen phosphorylase and cyclic AMP.[447]

Human chorionic gonadotrophin has been shown to bind to components of various sedimentable fractions from luteinized rat ovaries. Binding of the hormone and its asialo-derivative was most efficient, per unit weight of protein, when mitochondria and microsomes were used. Neither follicle-stimulating hormone nor thyrotrophin inhibited this reaction.[448]

Asialo human chorionic gonadotrophin was found to be as effective as the native hormone in bringing about morphological luteinization and in stimulating progestin secretion in cultured granulosa cells of rhesus monkeys, thus confirming that sialic-acid residues are not needed for this biological activity.[449]

Release of thyrotrophin from the anterior pituitary gland of chickens by the action of thyrotrophin-releasing hormone has been described.[450] There is a bimodal response, suggesting that the releasing hormone not only stimulates the release of stored thyrotrophin but also that of newly-synthesized hormone. Injection of thyrotrophin-releasing hormone into a hypothyroid woman has been reported to lead to the appearance of free α-chain within the serum after a few minutes.[451] Other workers have reported the appearance in serum of the β-subunit of thyrotrophin.[452] The β-subunit of human pituitary thyrotrophin consists of 112 amino-acid residues, and confirmation has been obtained that it carries a single carbohydrate moiety at asparagine-23, which occurs in the sequence -Asn-Thr-Thr-.[453]

Erythropoietin, an acid glycoprotein isolated from human urine, has been shown to contain hexose (13%), hexosamine (8.9%), and sialic acid (7.5%).[454] Its similarity in composition to human Tamm–Horsfall glycoprotein has been pointed out.[455]

Sugar compositions of the thyroglobulins from a range of species were found, not unexpectedly, to be somewhat different. Those from an amphibian (*Xenopus laevis*) and pig do not contain 2-amino-2-deoxy-D-galactose, which is found in thyroglobulins from chicken, whale, monkey, and man.[456] Thyroglobulin contains both small and large carbohydrate moieties, termed type-A and -B units, respectively;[457] the former is composed of

[447] L. M. Demers, S. G. Gabbe, C. A. Villee, and R. O. Greep, *Biochim. Biophys. Acta*, 1973, **313**, 202.
[448] B. J. Danzo, *Biochim. Biophys. Acta*, 1973, **304**, 560.
[449] C. P. Channing and S. Kammerman, *Endocrinology*, 1973, **93**, 1035.
[450] W. R. Breneman and W. Rathkamp, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 189.
[451] R. Benveniste, J. Bell, J. Koeppell, and D. Rabinowitz, *J. Clin. Endocrinol. Metab.*, 1973, **37**, 822.
[452] I. A. Kourides, B. D. Weintraub, E. C. Ridgway, and F. Maloof, *J. Clin. Endocrinol. Metab.*, 1973, **37**, 836.
[453] W. R. Sairam and C. H. Li, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 426.
[454] J. Espada, A. A. Langton, and M. Dorado, *Biochim. Biophys. Acta*, 1972, **285**, 427.
[455] A. M. S. Grant and A. Neuberger, *Biochem. J.*, 1973, **136**, 659.
[456] N. Ui, *J. Biochem. (Japan)*, 1973, **74**, 593.
[457] R. G. Spiro, *J. Biol. Chem.*, 1965, **240**, 1603.

D-mannose and 2-amino-2-deoxy-D-galactose, whereas the latter also contains other types of sugar, including L-fucose, sialic acid, and D-galactose. The type-B prosthetic group of pig thyroglobulin[458] and the type-A unit of the cattle glycoprotein[459] have each been shown to contain a β-linked D-mannosyl residue in the core region.

The overall conformation of thyroglobulin appears to be important in the capacity of the protein to form thyroxine upon iodination, but it is not clear how the sugars in the glycoprotein affect the stereochemistry of the product.[460] Differences in conformation of human thyroglobulin have been noted in healthy persons and in those with nodular goitre; these differences may be related to differences in the carbohydrate moieties.[461]

Thyroid-gland slices rapidly take up 2-amino-2-deoxy-D-glucose and incorporate it as 2-acetamido-2-deoxy-D-glucose and sialic acid into thyroglobulin. Incubation of cattle thyroid-gland slices with tracer 2-amino-2-deoxy-D-[^{14}C]glucose permitted an assessment of the amount of incorporation of this sugar into acid-soluble, hexosamine intermediates. Relatively large amounts were present as UDP-2-acetamido-2-deoxy-hexoses.[462]

Milk Glycoproteins

The lactose synthetase modifier protein, α-lactalbumin, exists in two genetic-variant forms, A and B, each of which consists of a major component (M) and three minor components (F, S_1, and S_2). Only S_1 and S_2 appear to be glycoproteins; it has been suggested these are glycosylated at asparagine-45, where the sequence is -Asn-Gln-Ser-.[463] The proposal [1] that the sequence glycosylated is -Asn-Ile-Ser- was made in error, and arose for typographical reasons. Messenger RNA for the α-lactalbumin of guinea-pig milk has been detected in mammary tissue, and now the control of glycoprotein synthesis at this stage may be examined.[464]

β-Lactoglobulin occurs in a number of genetic variants; two of them, A and Droughtmaster, appear to differ only in that the latter is a glycoprotein. From knowledge of the amino-acid sequence of the A variant of β-lactoglobulin[465] and of the composition of a tryptic glycopeptide (shorter than might have been expected) derived from the Droughtmaster variant,[466, 467] and from the likelihood that the linkage in a glycoprotein

[458] S. Toyoshima, M. Fukuda, and T. Osawa, *Biochem. Biophys. Res. Comm.*, 1973, **51**, 945.
[459] A. L. Tarentino, T. H. Plummer, and F. Maley, *J. Biol. Chem.*, 1973, **248**, 5547.
[460] M. Rolland, M.-F. Montfort, and S. Lissitzky, *Biochim. Biophys. Acta*, 1973, **303**, 338.
[461] Y. K. Turakulov, T. Saatov, P. A. Khakimov, and M. Vangibaer, *Biokhimiya*, 1972, **37**, 1113.
[462] J. L. Trujillo and J. C. Gan, *Biochim. Biophys. Acta*, 1972, **286**, 330.
[463] K. E. Hopper and H. A. McKenzie, *Biochim. Biophys. Acta*, 1973, **295**, 352.
[464] P. N. Campbell, D. McIlreavy, and D. Tarin, *Biochem. J.*, 1973, **134**, 345.
[465] G. Braunitzer, R. Chen, B. Schrank, and A. Stangl, *Z. physiol. Chem.*, 1972, **353**, 832.
[466] K. Bell, H. A. McKenzie, W. H. Murphy, and D. C. Shaw, *Biochim. Biophys. Acta*, 1970, **214**, 427.
[467] W. H. Murphy, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 115.

involving 2-acetamido-2-deoxy-D-glucose will also involve aspartic acid or asparagine residues,[1] it may be deduced that the Droughtmaster variant has a glycosylated asparagine residue at position 28 of the polypeptide chain, where the sequence in variant A is -Asp-Ile-Ser-. The fundamental difference between variants A and Droughtmaster is likely, therefore, to result from a single-base mutation, because the codons for asparagine are AAU and AAC and those for aspartic acid are GAU and GAC. Only if the Droughtmaster variant contained Asn at the analogous position would it undergo glycosylation. This deduction is in agreement with an assignment of the carbohydrate moiety to a region involving residues 15—40.[468] The Droughtmaster variant exhibits a number of unique properties due to its carbohydrate moiety; it does not octamerize at pH 4.7 and low temperature, as does the A-form. Moreover, the two forms differ in antigenicity.[466] The Droughtmaster variant was indicated to contain also a non-glycosylated sequence of the form -Asn-Pro-Thr-, which may have the form of a sequon for 2-acetamido-2-deoxy-D-glucose, but not act as an acceptor.[1]

κ-Casein is another example of a protein that is partly (28%) glycosylated.[469] Larger micelles of casein in cow's milk contain less total κ-casein than do the smaller micelles, although a greater proportion of the κ-casein is glycosylated. It has been proposed that glycosylated κ-casein is located inside the casein micelle and that the carbohydrate-free form is confined to the surface of the micelle.[470]

Certain aspects of the structure of the glycosylated form of κ-casein are mentioned earlier in this Report.[17, 19, 20] The total amino-acid sequence of bovine κ_B-casein (169 residues) has been described.[471] The A genetic variant has two different amino-acid substitutions in the caseinomacropeptide region,[471] one of them located within about three residues of a glycosylated threonine moiety.[19, 471] The sequence of para-κ_A-casein has also been reported;[472] the only differences found from κ_B-casein were in the substitution of glutamic acid for glutamine, and *vice versa*, and of asparagine for aspartic acid. The antigenic cross-reactivity of the κ_A-caseinoglycopeptides[20] with MN blood-group substances reflects similarities of the structures of the carbohydrate moieties.

One of several acid glycoproteins present in human colostrum has been isolated and characterized. It has a molecular weight of 3.12×10^4 and contains galactose (27%), hexosamine (21.7%), fucose (8.0%), and sialic acid (10.8%). The carbohydrate–peptide linkages were shown to involve

[468] D. C. Shaw, in 'Atlas of Protein Sequence and Structure', ed. M. O. Dayhoff, National Biochemical Research Foundation, Georgetown University Medical Center, Washington, D.C., Vol. 5, Suppl. 1, 1973, p. 5.

[469] D. G. Schmidt, P. Both, and P. J. De Koning, *J. Dairy Sci.*, 1966, **49**, 776.

[470] L. K. Creamer, J. V. Wheelock, and D. Samuel, *Biochim. Biophys. Acta*, 1973, **317**, 202.

[471] J.-C. Mercier, G. Brignon, and B. Ribadeau-Dumas, *European J. Biochem.*, 1973, **35**, 222.

[472] J. Jollés, F. Schoentgen, C. Alais, and P. Jollés, *Chimia* (*Switz.*), 1972, **26**, 645.

2-acetamido-2-deoxy-D-galactose and threonine, and the glycoprotein is devoid of ABH and MN activities.[473]

Other soluble sugar-transferases, presumably involved in the biosynthesis of glycoproteins, have been isolated from colostrum; *viz.* a sialyltransferase from goat, human, and cow colostra[474] and a 2-acetamido-2-deoxyglucosyltransferase from goat colostrum.[475] The reason for their presence in a soluble form is unknown. Another sialyltransferase was found in a particulate fraction of rat mammary gland.[476]

Galactosyltransferase, present in soluble form in cow's milk, has been shown to exist in two forms, of different molecular weight (5.8×10^4 and 4.5×10^4), each containing carbohydrate. From control experiments carried out with trypsin, the suggestion has come that the form of lower molecular weight is derived by a trypsin-like cleavage of the larger form.[477]

There appear to be glycoproteins with relatively high levels of sialic acid in echidna milk, and glycoproteins with high levels of L-fucose in the milk of the platypus.[478]

Serum Glycoproteins

The nature and metabolism of all serum glycoproteins are not yet fully known. Thus, the α_1-acute phase globulins of rat serum occur as a family of closely related proteins; some, but not all, of the variations reside in differences either in the content of sialic acid or in its manner of substitution.[479] Although albumin (to which particular asialoglycopeptides had been covalently linked *in vitro*) was rapidly cleared from circulation, the normal mechanism of catabolism of this protein is unknown.[480] The effects of intravenous neuraminidase on the turnover of fibrinogen have been studied.[481] Transferrin in serum has been shown to originate not only from the liver and lymphoid tissues, but also from the submaxillary gland, lactating mammary gland, ovaries, and testes.[482] However, the implications for glycosylation of this iron-binding protein remain to be established. By contrast, haemopexin appears to be formed only in the liver; a new procedure for its isolation has been described.[483]

The fact that the level of protein-bound carbohydrates increases during localized inflammation and systemic infection has been known for many

[473] J. H. Nichols and A. Bezkorovainy, *Biochem. J.*, 1973, **135**, 875.
[474] B. A. Bartholomew, G. W. Jourdian, and S. Roseman, *J. Biol. Chem.*, 1973, **248**, 5751.
[475] I. R. Johnston, E. J. McGuire, and S. Roseman, *J. Biol. Chem.*, 1973, **248**, 7281.
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[479] A. H. Gorden and P. J. Sykes, *Biochem. J.*, 1972, **130**, 95.
[480] J. C. Rogers and S. Kornfeld, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 622.
[481] E. Regoeczi and K.-L. Wong, in 'Protein Turnover', ed. G. E. W. Wolstenholme and M. O'Conner, Associated Scientific Publishers, Amsterdam, 1973, p. 181.
[482] G. J. Thorbecke, H. H. Liem, S. Knight, K. Cox, and U. Muller-Eberhard, *J. Clin. Invest.*, 1973, **52**, 725.
[483] A. Hayem-Levy and R. Havez, *Clinica Chim. Acta*, 1973, **47**, 113.

years; increases in the levels of α_1-, α_2-, and β-glycoglobulins, despite severe starvation, have also been observed.[484] Increases in the levels of orosomucoid, ceruloplasmin, haptoglobulin, and haemopexin specifically have been reported in hyperlipoproteinaemic patients.[485]

A large-scale fractionation procedure has been described for the preparation of orosomucoid,[486] and it has been shown that the properties of orosomucoid in solution are dramatically altered by removal of the sialic acid residues.[487] The five carbohydrate moieties of human orosomucoid are located in the *N*-terminal portion of the polypeptide chain, which contains 181 amino-acid residues. Homology between the sequences of amino-acids in this glycoprotein and in parts of the K-type light-chains and γ-type heavy-chains has led to the proposal that orosomucoid is related to an ancestral immunoglobulin.[488]

Factor-B activity of the properdin system is now known to be identical with a glycine-rich β-glycoprotein that does not react directly with a protein from cobra venom to produce a C3-inactivating complex.[489]

Lamb fetuin, isolated by a new procedure, is closely similar in composition to that obtained from calves,[490] and foetal mouse palate contains a plasma glycoprotein analogous to that of calf serum.[491]

A previously unknown α_1-macroglobulin has been isolated from human serum; it has a molecular weight of 3.08×10^5, contains 12% carbohydrate, and binds to metal ions (especially Ca^{2+} and Ni^{2+}). Human serum contains 55 mg of this macroglobulin per 100 ml.[492]

Just why animal cells grown in culture require specific sera is still not clear. Thus, the iron requirement of Chinese hamster V79 fibroblasts is met more efficiently by human transferrin than by either bovine transferrin or conalbumin. This is presumably due, in part at least, to the much greater ability of the former protein to bind to these cells, although differences in the nature of the transferrins (see below) may be responsible.[493]

There is another interesting effect resulting from the presence of serum in cultured cells: the type of sialic acid found in HeLa cells is dependent on the type of serum used in support of growth. *N*-Glycolylneuraminic acid occurred when calf serum was used, whereas only *N*-acetylneuraminic acid was present when human serum was added.[494]

[484] G. L. Cockerell, *Proc. Soc. Exp. Biol. Med.*, 1973, **142**, 1072.
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[494] L. Hof and H. Faillard, *Biochim. Biophys. Acta*, 1973, **297**, 561.

L-Fucose is a good marker for the synthesis of plasma glycoproteins in the Golgi complex in liver cells. From autoradiographic studies, it has been suggested that glycoproteins are exported from the Golgi complex in vesicles to plasma membranes for secretion from the liver.[495]

Soluble forms of a number of sugar transferases have been identified, including a number in sera. Two distinct fucosyltransferases have been demonstrated in sera: one of them transferred L-fucosyl residues to non-reducing, terminal 2-acetamido-2-deoxy-D-glucosyl residues in asialo-agalacto-orosomucoid and the other to the terminal D-galactosyl residues of lactose, *N*-acetyl-lactosamine, and asialo-orosomucoid. Individuals of the Bombay phenotype lack the second, but not the first, enzymic activity.[496]

A useful review on the chemistry and significance of the carbohydrate moieties of human-serum glycoproteins has described a number of their properties.[497]

Human α_1-antitrypsin has been isolated from sera using conventional chromatographic procedures,[498] as well as by the affinity-chromatography methods mentioned earlier.[86, 87] The former procedure yielded a material (molecular weight 5×10^4) containing D-mannose (7), D-galactose (5), 2-amino-2-deoxy-D-glucose (10), and sialic acid (6 residues per mole).[498]

α_1-Foetoprotein is produced by foetal liver and is normally present in serum up to two weeks after birth.[499] This glycoprotein, which binds oestrogen strongly, is also produced by primary liver cancer, and its presence in serum is of possible diagnostic value.[500] A procedure for isolating α_1-foetoprotein from human-cord blood, involving the use of Sepharose-linked concanavalin A, has been described.[501] α_1-Foetoprotein exists in more than one form in several species;[502, 503] one form, present both in rat foetal serum and in the serum of a patient with hepatoma, did not bind to concanavalin A–agarose.[504] An analogous glycoprotein from rabbit can be purified by immunochemical methods.[505]

The degree of sialylation of mouse α_1-foetoprotein in foetal plasma and in amniotic fluid increases with the length of pregnancy, the former more quickly. In each case, the amount of sialic acid in the glycoprotein is directly related to the level of sialyltransferase in the foetal liver and in the yolk sac, respectively.[506]

[495] J. M. Sturgess, E. Minaker, M. M. Mitranio, and M. A. Moscarello, *Biochim. Biophys. Acta*, 1973, **320**, 123.
[496] J. R. Munro and H. Schachter, *Arch. Biochem. Biophys.*, 1973, **156**, 534.
[497] K. Heide and H.-G. Schwick, *Angew. Chem. Internat. Edn.*, 1973, **12**, 721.
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[505] H. Pihko, J. Lindgren, and E. Ruoslahti, *Immunochemistry*, 1973, **10**, 381.
[506] E. F. Zimmerman and M. M. Madappally, *Biochem. J.*, 1973, **134**, 807.

An enzyme-linked (alkaline phosphatase) immunoassay has been applied in assessment of amounts of α_1-foetoprotein of the order of 1 ng per ml, and it is claimed to have many advantages over radioimmunoassay.[507]

An autoradiographic method, utilizing histological methods with tritiated oestrogens, gave results suggesting that α_1-foetoprotein is localized in hepatocyte-like cells and hepatocellular carcinomas. The cell producing the glycoprotein is believed to be distinct from either normal or neoplastic hepatocytes.[508]

A new, one-step procedure for the purification of human α_2-macroglobulin, using only 10 ml samples of blood, has permitted the analysis of the amino-acid and carbohydrate contents of the protein from individuals. Sugar contents {glucosamine (4.25%), hexoses (4.6%), and sialic acid (1.7%)} were the same in the glycoproteins of normals and patients,[509] although the overall level of glycoprotein was reported to be lower in juvenile rheumatoid arthritis (~350 mg per 100 ml) than in normal children (~430 mg per 100 ml).[510] Human α_2-macroglobulin bound to and inhibited the action of a number of substances including urokinase,[511] collagenase,[512] human cathepsin B1,[513] elastase,[514] trypsin and plasmin,[515] and many proteinases,[513, 516] including those from *Pseudomonas aeruginosa*.[517] The inhibitory activity was reported to involve cleavage of a peptide bond in α_2-macroglobulin, resulting in a change of conformation and trapping of the proteinase. The ways in which the sugars affect the activities of α_2-macroglobulin are unknown, and hypotheses concerning the inhibitory activities of this glycoprotein have been adumbrated.[518, 519] α_2-Acute-phase glycoprotein in rat sera appears to be analogous to α_2-macroglobulin in its ability to interact with trypsin and plasmin.[515, 520]

α_2-Macroglobulin has been found to be catabolized more rapidly in patients with burns.[521] This glycoprotein is dependent on its D-galactose residues, which are normally cryptic, for catabolism, but the involvement of this sugar in the glycoproteins of patients with burns has not been explored.

Although the serum transferrins from cattle, rabbit, horse, and pig each consist of a single polypeptide chain, differences have been observed in the

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[515] K. Ganrot, *Biochim. Biophys. Acta*, 1973, **322**, 62.
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[520] K. Ganrot, *Biochim. Biophys. Acta*, 1973, **295**, 245.
[521] S. P. Farrow and S. Baar, *Clinica Chim. Acta*, 1973, **46**, 39.

numbers of carbohydrate moieties which they contain. Those from cattle and chicken have one carbohydrate unit, those from rabbit, horse, and man have two, whereas pig transferrin has four heterosaccharide units.[522] These differences may be related to the occurrence of a sequence necessary for 2-acetamido-2-deoxy-D-glucosylation. The sequence of amino-acids (14 residues) has been determined in the region of one of the glycosylated asparagine residues in the protein from man.[523] Each of the two atoms of ferric iron binding to transferrin is accompanied by the binding of one bicarbonate ion, for which the carbohydrate moieties may be needed.[524]

Serum lipoproteins have been shown to contain carbohydrate. There is an accumulation in liver of the apoproteins for serum lipoproteins in rats fed on an adenine-free diet containing added orotic acid. The apoproteins, found in the rough endoplasmic reticulum, did not appear to have undergone complete glycosylation. It was proposed that the proteins had not been exposed to the glycosylating enzymes of the Golgi apparatus, and that the missing carbohydrate is a result of inhibition of the secretion of lipoprotein rather than its cause.[525] Less information is available on the conversion of the apoprotein into the lipoprotein. It is not known whether a covalent linkage in the form of an acylglyceryl cysteine residue, which occurs in some bacterial lipoproteins,[526] exists or whether formation of such a linkage and glycosylation are interdependent.

A new method for determining coagulation factor VIII in the presence of fibrinogen has been described.[527]

Factor X (Stuart factor) plays a key role in the formation of blood clots, and activation of the zymogen form of factor X generates the enzyme needed for converting prothrombin into thrombin. Cattle factor X has been separated into two forms (X_1 and X_2), each of which is composed of one heavy (molecular weight 3.9×10^4 or 3.7×10^4) and one light (molecular weight 1.9×10^4 or 1.7×10^4) polypeptide chain, respectively.[528, 529] It is reported that the heavy chains contain all the carbohydrate, and that X_1 and X_2 differ in having 22 and 27 monosaccharide residues, respectively.[528] Another report has claimed that the sugar compositions of X_1 and X_2 are identical, with about 14% of carbohydrate in the light chain in each case.[529] Treatment of Stuart factor X_1 with viper venom led to the release of a glycopeptide (molecular weight 1.1×10^4) from the *N*-terminus of the heavy chain, and to the formation of factor X_{1a}. A small amount of

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[529] K. Fujikawa, M. E. Legaz, and E. W. Davie, *Biochemistry*, 1972, **11**, 4882.

carbohydrate (5 residues) remained attached to factor X_{1a} in the region of the heavy chain still linked to the light chain.[530] Factor X_{1a} appears to be a glycosylated serine protease.[530, 531]

Degradation of factor X from pig serum, with concomitant activation, seemed to occur when it was adsorbed on to high-density barium sulphate. The clotting factor, which is produced in part, had a molecular weight of 3.9×10^4, as assessed by gel filtration using dextran markers.[532]

Desialized human prothrombin has been used as an exogenous acceptor for a sialyltransferase obtained in partially purified form from rat liver. The enzyme level was largely unchanged in the livers of rats made hypothrombinaemic by a diet deficient in vitamin K.[533] Horse prothrombin has been purified from citrated blood plasma by conventional procedures.[534]

The structure of thrombin is complex.[535–537] No evidence was found, according to one report,[535] for a form of human thrombin containing three, disulphide-linked, polypeptide chains reported in other studies.[536, 537] Only one (α) of three active forms of thrombin (α, β, and γ) was found to contain carbohydrate.[536]

Further studies have been carried out on the antifreeze glycoproteins present in some species of Antarctic fish. The mechanism of their action does not involve supercooling,[538] but probably involves formation of a coating on the surface of ice crystals, which then no longer act as sites of nucleation.[539, 540] An antifreeze protein has also been found in serum of the winter flounder, *Pseudopleuronectes americanus*, the nature of which may be similar to, but not identical with, that of Antarctic fish.[541]

The serum of the bullfrog, *Rana catesbeiana*, has been shown to contain an α_1-glycoprotein that increases in concentration during metamorphosis (30 mg % in the tadpole to 300 mg % in the adult). It has a molecular weight of *ca.* 2.0×10^5 and contains 10% carbohydrate.[542]

The secretion of serum glycoproteins and albumin may involve the same pathway,[543] although it was suggested that the former are membrane-bound, whereas the latter is not.[544] Orosomucoid is produced at an increased rate in livers of animals with inflammation.[545]

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[539] J. A. Raymond and A. L. DeVries, *Cryobiology*, 1972, **9**, 469.
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Immunoglobulins

Antibodies can be usefully prepared with the aid of antigens coupled to cellulose cyclic carbonate. Rabbit IgG and human IgM have been used as antigens in the preparation of antibodies from sheep sera.[546] Messenger RNA(14*S*) for a possible precursor of a mouse light chain has been purified from myeloma cells.[547]

The structures of immunoglobulins and the relationship of structure to activity were discussed in the Nobel lectures of R. R. Porter[548] and G. M. Edelman.[549] Fruit bromelain cleaved IgG into fragments closely similar to those obtained by papain digestion, but certain of the subclasses resisted the action of the enzyme.[550] No carbohydrate was found to be attached to the C-region of the F_d fragment in a rabbit IgG allotype a2, in accord with earlier findings on pooled IgG.[551] Chicken anti-DNP IgG and pooled IgG were shown, by isotope-dilution assays, to contain equal amounts of carbohydrate. IgG from this species is relatively rich in carbohydrate (30 residues of hexosamine, 3.4 residues of D-galactose, and 28 residues of D-mannose per molecular weight of 1.7×10^5).[552] Human secretory IgA (S-IgA) has its carbohydrate residues distributed between the four α-chains (100 residues of sugar), the secretory component (45 residues), and the single J-chain (10 residues).[553] The molecular weight of the J-chain isolated from a human myeloma A-protein was found to be 1.56×10^4.[554]

The immune macroglobulin from the sera of channel cat-fish, *Ictalurus punctatus*, has been shown to be a tetramer containing 5.5% carbohydrate. The immunoglobulin was reported to contain D-mannose, D-galactose, L-fucose, sialic acid, and 2-acetamido-2-deoxy-D-galactose, but no 2-acetamido-2-deoxy-D-glucose.[555]

Isolation and characterization of the immunoglobulins of sharks[556] and of IgG 2 of hedgehogs[557] have been described; the latter contains 2% carbohydrate.

The sequence of amino-acids adjacent to one of the carbohydrate moieties linked to an asparagine residue in the α-chain of human myeloma A1 protein (Ha) has been reported to be of the form expected (Asn-Leu-Thr); the carbohydrate moiety contains neither L-fucose nor sialic acid residues.[558] It has been confirmed, using a plasma-cell tumour MOPC 315,

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[547] B. Mach, C. Faust, and P. Vassalli, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 451.
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[558] V. Moore and F. W. Putnam, *Biochemistry*, 1973, **12**, 2361.

that glycosylation of IgA occurs by the stepwise addition of carbohydrate during secretion. Control of IgA polymerization is almost certainly not regulated by the addition of carbohydrate.[559] Similarly, the addition of sialic acid does not control the secretion of mouse myeloma IgG protein (MOPC 21) from the plasmacytoma, since both intra- and extra-cellular forms of the glycoprotein are devoid of this sugar. The reason for differences in the electrophoretic mobilities of the two forms has still to be resolved.[560]

Six mouse myeloma IgA proteins with binding activity directed against β-(1 → 6)-D-galactose oligomers have been isolated from ascites fluid by affinity chromatography.[561] These proteins have different idiotypic determinants.[562] Two of the myeloma proteins can probably incorporate only three sugar residues into their binding sites, which are, therefore, rather small.[561]

An established line of human lymphoma cells (Daudi) has both IgM and complement on the cell surface; there are about 10^5 molecules of monomeric IgM per cell, and only free μ- and light-chains are present within the cell. Daudi cells are probably neoplastic analogues of B lymphocytes.[563]

Comparisons have been made between immunoglobulins on the cell surface of lymphocytes of normal subjects and those of cases of various infectious diseases[564] and agammaglobulinaemia and selective IgA deficiencies.[565]

An increased rate of production of 7*S* and 19*S* antibodies was evoked against suboptimal levels of sheep red-cell antigen, provided pentosan sulphate (molecular weight 2×10^3) was injected just before the antigen.[566]

Myeloma glycoprotein (Mcg) has been shown to have a deletion of residues 216—232 in the hinge region.[567] Another heavy-chain-disease protein (type γ4) contains a deletion of the gene(s) coding for residues 11 or 12—256. The protein is glycosylated.[568]

Further support for the role of immunoglobulins in the production of some types of amyloid is provided by the finding that limited proteolysis of certain Bence-Jones proteins, mainly those of the Vλ1 class, can lead to the

[559] E. Della Corte and R. M. E. Parkhouse, *Biochem. J.*, 1973, **136**, 589.
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formation of amyloid-like fibrils.[569, 570] An instance of a patient with generalized amyloidosis, involving a monoclonal IgG 2 K-type cryoglobulin, has been described.[571] Protein of the type of amyloid that is not of the immunoglobulin form may arise from an α_1-globulin.[572]

The relation of the ciliary factor in the sera of patients with cystic fibrosis to the postulated basic defects in the disease is unknown. The factor was found to bind strongly to immunoglobulins IgG_1 and IgG_2, and not to contain uronic acid.[573]

Blood Cellular Element Glycoproteins

The role of platelets in haemostasis and platelet-abnormalities has been discussed in relation to heritable disorders of connective tissues.[574] Lactoperoxidase-catalysed iodination of human-platelet membrane resulted in labelling predominantly of a membrane protein with a molecular weight of *ca.* 1×10^5. The labelled glycoprotein was purified by extraction with lithium 3,5-di-iodosalicylate and by affinity chromatography on concanavalin A-agarose. Less labelling of the surface occurred when the platelets were first treated with neuraminidase.[575] It was observed in other studies that glycoproteins of the erythrocyte surface act as a barrier to lactoperoxidase-catalysed iodination.[576]

The α_1-chain of denatured, chicken-skin collagen has been shown to cause aggregation of platelets. The active part of the α_1-chain resides in a glycopeptide containing 36 amino-acid residues, including an amino-terminal *O*-α-D-glucopyranosyl-(1 → 2)-*O*-β-D-galactopyranosyloxy-(1 → 5)-lysine moiety. The sugar residues are involved in the induced aggregation of platelets.[577]

A non-ionic detergent, NP-40, has been used for separating the soluble histocompatibility antigens from human platelets; the active products have molecular weights in excess of 2×10^5.[578]

The predominant glycoprotein of cattle-erythrocyte membrane has a molecular weight of 1.8×10^5 and contains 62% carbohydrate.[579] It has been reported that at least three glycoproteins at the surface of human erythrocytes are exposed to the external environment. This was deduced

[569] R. P. Linke, D. Zucker-Franklin, and E. C. Franklin, *J. Immunol.*, 1973, **111**, 10.
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[579] R. A. Capaldi, *Biochim. Biophys. Acta*, 1973, **311**, 386.

from the results of gel electrophoresis in sodium dodecyl sulphate of red-cell ghosts previously treated with galactose oxidase and sodium borotritide.[580] Studies have also been made on the interaction of acid glycosaminoglycans with erythrocyte surfaces.[581]

Changes in the nature of the glycoproteins present in post-nuclear particulate fractions of neutrophil leucocytes occurred during maturation. A number of glycoproteins did not appear until later stages of maturation, whereas others disappeared.[582]

T-Lymphocytes have been shown to possess a glycoprotein coat that is nearly twice as dense as that on B-lymphocytes.[583]

Salivary, Mucous, and Other Mammalian Body-fluid Glycoproteins

Staining with Alcian Blue has proved useful not only for the detection of glycosaminoglycans, but also for differentiating between sialic-acid-containing and sulphate-containing mucins; the former will not stain at a pH < 1.5.[584] The procedure was used to examine the mucins of human bronchial submucosal glands.[585]

The difficulties of isolating the glycoproteins of normal bronchial mucin have been pointed out[586] and a number of rheological properties of mucus have been described.[587] Dibutyryl cyclic AMP appeared to increase the rate of release of mucus from human bronchial mucous cells.[588] Exposure to tobacco smoke led to an increase in the number of goblet cells of the respiratory epithelium and to a change in the nature of the glycoproteins formed by these cells.[589] Injections of isoprenaline and pilocarpine resulted in increased numbers of bronchial goblet cells, the former drug acting only on cells producing acid glycoproteins.[590] Neutral bronchial mucins, prepared from the fibrillar part of the sputum of a patient with chronic bronchitis, was found to contain relatively few amino-acids and 86% carbohydrate.[591] Certain features of these mucins are similar to those of glycoproteins isolated from bronchogenic cysts.[592]

The respiratory mucins of humans and dogs may have blood-group A activity. A 2-acetamido-2-deoxy-D-galactosyltransferase was shown to be

[580] C. G. Gahmberg and S.-I. Hakomori, *J. Biol. Chem.*, 1973, **248**, 4311.
[581] S. M. Bychkov and S. A. Kuzmina, *Biull. Eksp. Biol. Med.*, 1973, **75**, 40.
[582] F. L. Huang and W. H. Evans, *Biochim. Biophys. Acta*, 1973, **317**, 394.
[583] V. Santer, R. E. Cone, and J. J. Marchalonis, *Exp. Cell Res.*, 1973, **79**, 404.
[584] R. Jones and L. Reid, *Histochem. J.*, 1973, **5**, 9.
[585] R. Jones and L. Reid, *Histochem. J.*, 1973, **5**, 19.
[586] L. Reid, *Bulletin de Physio-pathologie Respiratoire*, 1973, **9**, 15.
[587] L. Reid, *Scientific Basis Med. Ann. Revs.*, 1973, 130.
[588] W. F. Whimster and L. Reid, *Exp. Mol. Pathol.*, 1973, **18**, 234.
[589] R. Jones, P. Bolduc, and L. Reid, *British J. Exp. Pathol.*, 1973, **54**, 229.
[590] J. Sturgess and L. Reid, *British J. Exp. Pathol.*, 1973, **54**, 388.
[591] G. Lamblin, M. Lhermitte, P. Dégand, Y. H. Sergeant, and P. Roussel, *Biochim. Biophys. Acta*, 1973, **322**, 372.
[592] P. Dégand, P. Roussel, G. Lamblin, and R. Havez, *Biochim. Biophys. Acta*, 1973, **320**, 318.

present in a particulate preparation from A-active dog trachea; the enzyme converted human blood-group H substance into A substance.[593] A deficiency of vitamin A greatly reduced, whereas retinoic acid stimulated, the uptake of L-fucose into the glycoproteins of hamster trachea. These processes appear to involve at least one specific glycopeptide, or family of glycopeptides, which has been isolated from the trachea.[594]

Explants of dog trachea have been used in studies on the formation of glycoproteins, and exogenous 2-amino-2-deoxy-D-glucose has been found to inhibit the incorporation of L-fucose and serine into the glycoproteins, which were shown, by electrophoresis in agarose gels, to be identical with those produced *in vivo* by the respiratory tract.[595] Using similar techniques, it has been demonstrated that there are differences in the mucous secretions produced by epithelial goblet cells and submucosal glands; those secreted by the former being more extensively sulphated.[596] The sulphated glycoproteins produced by submucosal glands, at least in man, appear to be of three classes: *viz.* those that stain histochemically both at pH 2.6 and 1.0 with Alcian Blue, those that stain only at pH 1.0, and a third group that stains only with the iron diamine technique.[585]

Occurrence of repetitive sequences of amino-acids in the submaxillary-gland glycoproteins of cattle has been confirmed.[597] The predominant glycoprotein produced by the submaxillary gland of the horse has been shown to possess a high molecular weight ($> 10^6$); it contains large amounts of 2-amino-2-deoxy-D-galactose and also 2-amino-2-deoxy-D-glucose, D-galactose, L-fucose, and sialic acid. Many of the carbohydrate–peptide linkages are of the type involving serine and threonine.[598]

A sialyltransferase present in sheep submaxillary gland has been shown to catalyse the transfer of sialic acid from CMP-sialic acid to an acceptor prepared by treating sheep submaxillary-gland glycoprotein with neuraminidase. Almost 70% of the sialic acid residues were transferred to C-6 of 2-acetamido-2-deoxy-D-galactosyl residues.[599]

A non-competitive inhibitor of β-glucuronidase has been isolated from the sublingual gland of the pig; it is a glycoprotein (molecular weight 3.4×10^5) containing hexose (20.8%), hexosamine (19.9%), fucose (9.6%), and *N*-acetylneuraminic acid (21.8%).[600] Its physiological role, if any, is not known, since β-glucuronidase was not inhibited by the glycoprotein at physiological ionic strength.

[593] A. P. Baker, L. J. Griggs, J. R. Munro, and J. A. Finkelstein, *J. Biol. Chem.*, 1973, **248**, 880.
[594] F. Bonanni, S. S. Levinson, G. Wolf, and L. De Luca, *Biochim. Biophys. Acta*, 1973, **297**, 441.
[595] D. B. Ellis and G. H. Stahl, *Biochem. J.*, 1973, **136**, 837.
[596] G. H. Stahl and D. B. Ellis, *Biochem. J.*, 1973, **136**, 845.
[597] W. Pigman, J. Moschera, M. Weiss, and G. Tettamanti, *European J. Biochem.*, 1973, **32**, 148.
[598] H. Huser, E. Mody, and H. Faillard, *Z. physiol. Chem.*, 1973, **354**, 749.
[599] D. M. Carlson, E. J. McGuire, G. W. Jourdian, and S. Roseman, *J. Biol. Chem.*, 1973, **248**, 5763.
[600] W. Sakamoto, O. Nishikase, and E. Sakakibara, *Biochim. Biophys. Acta*, 1973, **329**, 72.

The two glycoproteins capable of binding vitamin B_{12} have been isolated from pig gastric mucosa by affinity chromatography on a matrix containing the vitamin linked to agarose. The glycoproteins differed in their amino-acid and carbohydrate compositions, and only one of them functioned as an intrinsic factor (as judged by Schilling tests).[601] Intrinsic factor was also isolated from human gastric juice by a procedure that included affinity chromatography on a column of hydroxycobalamin-agarose. Many of the chemical, physical, and biological properties of this factor were studied.[602]

A glycoprotein fraction from sheep gastric mucosa contained 7—9% of its carbohydrate moieties in the form Neu-Gal-(2 → 6)-Gal*N*Ac-(1 → 3)-(?)-Gal and 20% in the form Fuc-(1 → 2 or 6)-Gal-(1 → 6)-Gal*N*Ac. An indication of the positions of mannose and 2-amino-2-deoxyglucose residues was obtained by Smith degradation.[603]

A glycopolypeptide isolated from pronase digests of human gastric mucosa contained large numbers of serine and threonine residues, which were shown to be glycosylated mainly with branched-chain carbohydrate moieties.[604] A number of the problems likely to be encountered in studies of mucous glycoproteins have been reviewed.[605]

The sedimentation behaviour of pig gastric mucus has revealed the presence of three fractions (21.5, 11.5, and 5.0*S*), probably in the form of aggregates.[606] The major glycoprotein (18% protein) of the mucus formed viscous solutions, the reduced, specific viscosities of which increased with both the concentration of glycoprotein and temperature; for example, a reversible sol–gel transition occurred at 25 °C with a concentration of 4 mg ml^{-1} and at 45 °C with a concentration of 2 mg ml^{-1}. The changes observed with temperature were ascribed to hydrophobic interactions between soluble glycoproteins.[607]

The viscosity of pig gastric mucin decreased at an acid pH (< 3.0) when the solution was titrated with hydrochloric acid; however, in the presence of H_4edta, the viscosity was observed to rise. It was suggested that Ca^{2+} ions are normally responsible for changes in viscosity.[608]

There appear to be a number of proteins and glycoproteins in gastric juice which bind iron in a non-specific way.[609, 610] The results of these studies have cast doubt on the presence therein of a specific, iron-binding glycoprotein, gastroferrin, which is known to have an amino-acid and

[601] R. H. Allen and C. S. Mehlman, *J. Biol. Chem.*, 1973, **248**, 3670.
[602] J. M. Christensen, E. Hippe, H. Oleson, M. Rye, E. Haber, L. Lee, and J. Thomsen, *Biochim. Biophys. Acta*, 1973, **303**, 319.
[603] R. Huguet, M. Solere, and N. Remy-Heintz, *Carbohydrate Res.*, 1972, **24**, 393.
[604] L. Hough and J. V. S. Jones, *Carbohydrate Res.*, 1972, **23**, 1.
[605] W. Pigman and J. Moschera, in 'Carbohydrates in Solution', Adv. in Chem. Series, Amer. Chem. Soc., 1973, Vol. 117, p. 220.
[606] P. Johnson and K. D. Rainsford, *Biochim. Biophys. Acta*, 1972, **286**, 72.
[607] D. Snary, A. Allen, and R. H. Pain, *European J. Biochem.*, 1973, **36**, 72.
[608] J. Deman, M. Mareel, and E. Bruyneel, *Biochim. Biophys. Acta*, 1973, **297**,486.
[609] C. H. J. Swan and G. B. Jerzy Glass, *J. Lab. Clin. Med.*, 1973, **81**, 719.
[610] A. Bella and Y. S. Kim, *Biochim. Biophys. Acta*, 1973, **304**, 580.

carbohydrate composition closely similar to that of the predominant glycoprotein of gastric juice.[1] The defect in haemochromatosis is therefore unknown.

Not unexpectedly, there may be differences in rats in A, B, and H blood-group activities manifest in red cells and in gastrointestinal mucins. Although all Sprague–Dawley rats appeared to be $A^+B^+H^+$ with respect to red cells, a large proportion (*ca.* 57%) were found to be of the type $A^-B^+H^+$ in the mucin produced in the small intestinal mucosa. This group of rats is deficient in a mucosal 2-acetamido-2-deoxy-D-galactosyltransferase that is present in rats which are $A^+B^+H^+$ with respect to the mucosa.[611]

There are three separate calcium-binding proteins present in bovine small intestine[612] and two in human duodenal mucosa.[613] One of the components present in bovine intestine has been reported to undergo changes on prolonged storage, with production of the other two. Bovine kidney also contains a calcium-binding protein that undergoes immunological cross-reaction with those in the intestine.[612]

Amniotic fluid of mice contains glycoproteins produced by the foetus, one of which is a transferrin distinct from that produced by the mother.[614]

Cow-oestrus cervical mucin has been shown to have an *N*-terminal alanine residue and it also contains cysteine; results of amino-acid analyses suggested a repeating, basic sequence of amino-acids, the unit being about 32—35 residues long.[615] A new analysis by g.l.c. was also described.[616]

Changes in the nature of the glycocalyx of human placenta occurring during maturation have been observed, although not fully investigated.[617]

Evidence presented suggests that a metabolic change in endometrial glycoproteins may play an important role in the infertility effect caused by intrauterine devices.[618] The precise nature of the glycoproteins has not been investigated.

The concentration of sialic acid covalently linked in glycoproteins in the vagina of rats increased after removal of the ovaries; on the other hand, the concentration decreased on subsequent intramuscular injection of oestradiol. The effects of other steroid hormones have also been studied.[619]

A rare example of a delayed metastasis of a mucus-producing cell carcinoma of the stomach to the skin has been described. The appearance of scleromyxoedema was caused by the production of mucus by the cutaneous metastatic cells, although the exact nature of the glycoproteins formed was not elucidated.[620]

[611] Y. S. Kim and J. Perdomo, *J. Clin. Invest.*, 1973, **51**, 1135.
[612] C. S. Fullmer and R. H. Wasserman, *Biochim. Biophys. Acta*, 1973, **317**, 172.
[613] D. H. Alpers, S. W. Lee, and L. V. Avioli, *Gastroenterology*, 1972, **62**, 559.
[614] D. L. Gustine and E. F. Zimmerman, *Amer. J. Obstet. Gynecol.*, 1972, **114**, 553.
[615] K. S. P. Bhushana Rao, E. Van Roost, P. L. Masson, J. F. Heremans, and F. André, *Biochim. Biophys. Acta*, 1973, **317**, 286.
[616] F. André, C. André, K. S. P. Bhushana Rao, P. L. Masson, and J. F. Heremans, *Carbohydrate Res.*, 1972, **25**, 395.
[617] R. A. Rovasio and B. Monis, *Experientia*, 1973, **29**, 1115.
[618] T. Abe, M. Endo, and Z. Yosizawa, *Clinica Chim. Acta*, 1972, **42**, 29.
[619] F. Galletti and R. Gardi, *J. Endocrinol.*, 1973, **57**, 193.
[620] E. J. Feuerman, M. A. Nir, and M. Lurie, *Dermatologica*, 1973, **146**, 15.

Urinary Glycoproteins and Glycopeptides

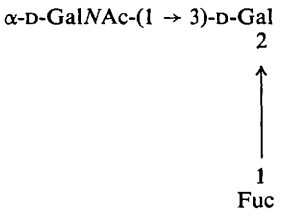
Patients with mannosidosis have been shown to excrete in their urine various D-mannose-containing oligosaccharides (5)—(7), which are probably

α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-D-Glc*N*Ac
(5)

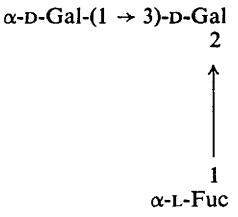
α-D-Man-(1 → 2)-α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-D-Glc*N*Ac
(6)

α-D-Man-(1 → 2)-α-D-Man-(1 → 2)-α-D-Man-(1 → 3)-β-D-Man-(1 → 4)-D-Glc*N*Ac
(7)

derived from glycoproteins.[621, 622] Other oligosaccharides have been isolated from the brain and urine of Angus cattle with mannosidosis; two tetrasaccharides possess structures of the form D-Man-D-Glc*N*Ac-D-Man-D-Glc*N*Ac, and differ from any so far isolated from the urine of humans with mannosidosis.[623] The disaccharides α-D-Xyl-(1 → 3)-D-Glc and α-L-Fuc-(1 → 3)-D-Glc were found [624] in normal urine, together with trisaccharides (8) and (9) that may have been derived from blood-group substances.[625]



(8)



(9)

[621] N. E. Nordén, A. Lundblad, S. Svensson, P.-A. Öckerman, and S. Autio, *J. Biol. Chem.*, 1973, **248**, 6210.
[622] N. E. Nordén, A. Lundblad, S. Svensson, and S. Autio, *Biochemistry*, 1974, **13**, 871.
[623] N. E. Nordén, A. Lundblad, P.-A. Öckerman, and R. D. Jolly, *F.E.B.S. Letters*, 1973, **35**, 209.
[624] A. Lundblad and S. Svensson, *Biochemistry*, 1973, **12**, 306.
[625] A. Lundblad, P. Hallgren, A. Rudmark, and S. Svensson, *Biochemistry*, 1973, **12**, 3341.

A glycopeptide isolated from the urine of pregnant women probably contains of a single heterosaccharide, containing 2-acetamido-2-deoxy-D-glucose, D-galactose, D-mannose, and sialic acid (six, seven, three, and four residues, respectively), linked to an asparagine residue in the amino-acid sequence -Asn-Thr-Ser-. The glycopeptide inhibited the binding of the lectin from *Robinia pseudacacia* to erythrocytes, hepatocytes, and Zajdela tumour cells. Release of sialic acid did not alter this activity.[626]

The development of a radioimmunoassay procedure for determining less than 5 μg quantities of urinary Tamm–Horsfall glycoprotein[627] has permitted accurate determination of the levels of this kidney product in normal adults and children and in patients with kidney diseases.[628] The Tamm–Horsfall glycoprotein in man has a half-life of about 16 hours; for rabbits it is about 9 hours.[455] The glycoprotein bound Ca^{2+} ions strongly; the extent of binding increased approximately linearly with the concentration of free Ca^{2+} ions until, at a maximum of 0.45 mmol Ca^{2+} per g glycoprotein, gel-formation occurred. Acid–base titration of the glycoprotein from humans was carried out in solutions of 6M-guanidinium chloride.[629] Sialic-acid-free T–H glycoprotein has been used as a substrate for kidney sialyltransferase. The reaction was inhibited by H_4edta, although sialyltransferases do not usually require metal ions.[630]

A number of carboxylic esterases have been isolated, by affinity chromatography using concanavalin A, from the urine of a man with cancer of the prostate. All were presumed to be glycoproteins.[631]

One of the urogastrones has been isolated from the urine of pregnant women, one litre of urine yielding 1.71 mg. It was shown to be a glycoprotein, containing 37% carbohydrate, composed of sialic acid, fucose, hexose, and hexosamine in molar ratios of 1.3 : 1 : 5 : 1.6. Its gastric antisecretory activity was not removed by treatment with neuraminidase.[632]

Avian-egg Glycoproteins

The purification of messenger RNA for hen egg-white albumin,[633] and the use of RNA-directed DNA-polymerase of Rous sarcoma virus to catalyse the production of DNA complementary to messenger RNA[634] should lead to information about the base sequences needed to produce the amino-acid sequence required for 2-acetamido-2-deoxy-D-glucosylation of the polypeptide chain.

[626] M. Lemonnier, Y. Goussault, and R. Bourrillon, *Carbohydrate Res.*, 1972, **24**, 323.
[627] A. M. S. Grant and A. Neuberger, *Clinical Sci.*, 1973, **44**, 163.
[628] A. M. S. Grant, L. R. I. Baker, and A. Neuberger, *Clinical Sci.*, 1973, **44**, 377.
[629] A. J. Cleave, P. W. Kent, and A. R. Peacocke, *Biochim. Biophys. Acta*, 1972, **285**, 208.
[630] B. B. Kirchbaum and H. B. Bosmann, *Nephron*, 1973, **11**, 11.
[631] T. C. Bøg-Hansen, H. Brogren, and M. Rostgaard, *I.R.C.S.*, 1973, **3-1**, 22.
[632] G. Carrea, M. M. Casellato, E. Manera, P. Pasta, and G. Lugaro, *Biochim. Biophys. Acta*, 1973, **295**, 274.
[633] R. Palacios, D. Sullivan, N. M. Summers, M. L. Kiely, and R. T. Schimke, *J. Biol. Chem.*, 1973, **248**, 540.
[634] D. Sullivan, R. Palacios, J. Stavnezer, J. M. Taylor, A. J. Faras, M. L. Kiely, N. M. Summers, J. M. Bishop, and R. T. Schimke, *J. Biol. Chem.*, 1973, **248**, 7530.

Immunoglobulin raised against hen's egg albumin, like the latter glycoprotein, contains a number of tyrosine residues having abnormally high pK_a values.[635] The fit of this immunoglobulin to egg albumin was not investigated nor was the interaction between lysozyme and egg albumin, which has now been studied by sedimentation equilibrium,[636] partly because the stereochemistry of albumin is unknown.

The neutral glycopeptides derived from hen ovomucoid have been subjected to acetolysis, and oligosaccharides (10)—(22) were among the products characterized after deacetylation.[637]

β-D-Glc*N*Ac-(1 → 2)-α-D-Man-(1 → 3)-D-Man
(10)

β-D-Glc*N*Ac-(1 → 2)-D-Man
(11)

β-D-Glc*N*Ac-(1 → 4)-D-Man
(12)

β-D-Gal-(1 → 4)-D-Glc*N*Ac
(13)

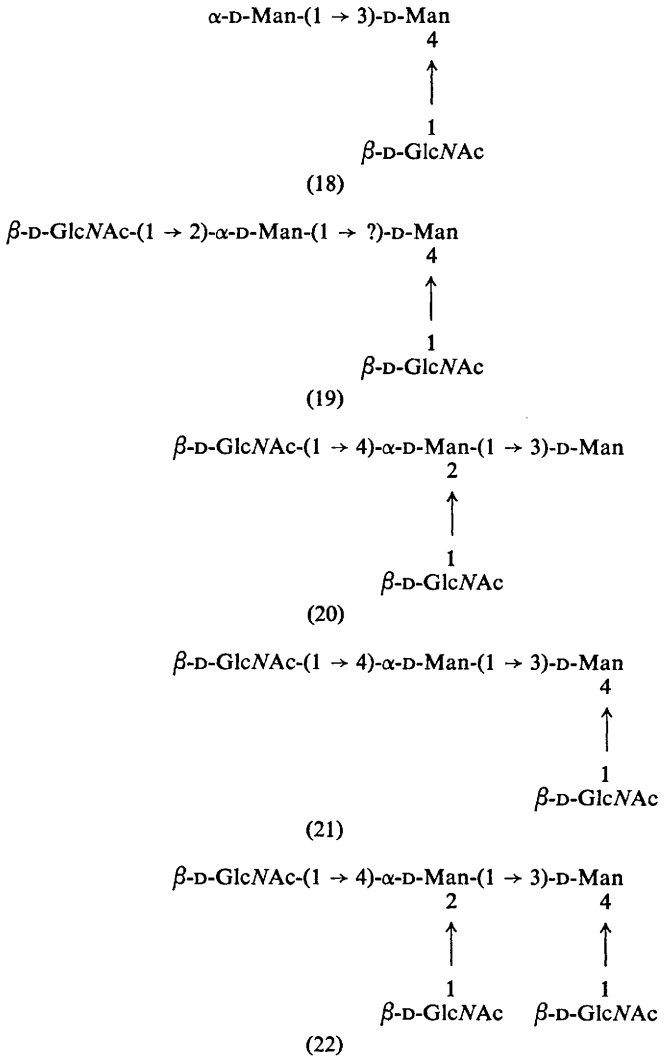
α-D-Man-(1 → 3)-D-Man
(14)

β-D-Glc*N*Ac-(1 → 4)-α-D-Man-(1 → 3)-D-Man
(15)

β-D-Glc*N*Ac-(1 → 3)-D-Man
2
↑
1
β-D-Glc*N*Ac
(16)

β-D-Glc*N*Ac-(1 → 4)-D-Man
2
↑
1
β-D-Glc*N*Ac
(17)

[635] A. A. Ansari and A. Salahuddin, *European J. Biochem.*, 1973, **35**, 290.
[636] G. J. Howlett and L. W. Nichol, *J. Biol. Chem.*, 1973, **248**, 619.
[637] B. Bayard, B. Fournet, S. Bouquelet, G. Strecker, G. Spink, and J. Montreuil, *Carbohydrate Res.*, 1972, **24**, 445.



The properties of the phosphoglycoprotein phosvitin, as determined by viscosity and chiroptical measurements on its solutions, have been shown to resemble those of a polyelectrolyte. It is difficult to see how the carbohydrate in this glycoprotein contributes to the β-structure observed at values of pH <3.6.[638]

Ovomucin can be isolated by a procedure involving high-speed centrifugation of thick egg-white made 0.5molar in potassium dihydrogen phosphate

[638] G. E. Perlmann, *Israel J. Chem.*, 1973, **11**, 393.

(pH 5.7). The yield and purity of the product obtained by this simplified process compared favourably with other preparations.[639] The relation of this product to three fractions of ovomucin, which have different solubilities in electrolyte solutions,[640] remains to be determined. Furthermore, β-ovomucin appeared to undergo conversion into a more soluble form during the natural thinning of thick egg-white.[641] An account of the way in which the sugar residues of the molecule are involved in the change is awaited with interest, since the carbohydrate content (35.0%) of ovomucin from thick egg-white has been reported to be larger than that (29.2%) from thin egg-white.[642]

Similarities in the structures and properties of sulphated glycopeptides derived from egg-shell membranes and hen oviduct have been discussed in relation to the suggestion that both magnum and isthmus in oviduct are concerned with synthesis of the glycoproteins of egg-shell membranes.[643]

Miscellaneous Glycoproteins

The dermis of the sea cucumber *Thyone briareus* has been shown to contain a glycoprotein in which the carbohydrate moieties consist largely of (1 → 2)-linked α-L-fucopyranosyl 4-sulphate residues and small amounts of D-galactose; it has been named thyonatan 4-sulphate.[644] The nature of the carbohydrate–peptide linkage is unknown. A hexosamine-containing poly(L-fucose sulphate)–protein complex has been found in the sea cucumber *Stichopus japonicus* Selenka.[645] L-Fucose 4-sulphate was also isolated from acid hydrolysates of the egg-jelly glycoproteins of two kinds of sea urchins, *viz. Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus*.[646]

The eggs of grasshoppers contain a mannan-like polysaccharide bound to protein, possibly by a linkage between 2-acetamido-2-deoxy-D-glucose and L-asparagine residues.[647] A disaccharide, probably of structure L-Fuc-(1 → 4)-Neu*N*Gl, has been isolated from mild acid hydrolysates of the jelly coat of eggs of the sea urchin *Pseudocentrotus depressus* and may have arisen from a glycoprotein.[648]

A factor involved in aggregation of the marine sponge *Microciona parthena* has been recognized as a glycoprotein containing 49% carbo-

[639] R. W. Sleigh, G. J. H. Melrose, and M. B. Smith, *Biochim. Biophys. Acta*, 1973, **310**, 453.
[640] M. L. Lyndnup, *Prep. Biochem.*, 1973, **3**, 135.
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[643] J. Picard, A. Paul-Gardais, and M. Vedel, *Biochim. Biophys. Acta*, 1973, **320**, 427.
[644] R. L. Katzman and R. W. Jeanloz, *J. Biol. Chem.*, 1973, **248**, 50.
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[646] K. Ishihara, K. Oguri, and H. Taniguchi, *Biochim. Biophys. Acta*, 1973, **320**, 628.
[647] K. Yamasaki, *Insect Biochem.*, 1973, **3**, 79.
[648] K. Hotta, M. Kurokawa, and S. Isaka, *J. Biol. Chem.*, 1973, **248**, 629.

hydrate; galactose, mannose, uronic acid, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose were identified among the sugars present.[649] The isolated glycoprotein is a large, fibrous molecule having a molecular weight of 2.1×10^6 in the presence of Ca^{2+} ions; when the ions were removed, dissociation into particles of molecular weight 2×10^5 occurred.[650]

Eight glycoproteins have been found in or near the surface of the trypanosome; a complex of these glycoproteins is antigenic.[651]

A glycoprotein containing sialic acid was reported to be present in the mucus produced by the goblet cells of the epidermis of the eel *Anguila japonica*. Its behaviour is similar to that of glycoproteins produced by mammalian epithelial tissue.[652]

The biochemical basis for the genetic heterogeneity in fucosidosis is unknown.[653, 654]

The glycolipophosphoprotein vitellogenin is secreted *in vitro* by liver slices from oestrogen-treated *Xenopus laevis* (the South African clawed toad). A delay of about 2 hours occurred before the radiolabelled leucine or phosphate supplied to the slices appeared in the secreted vitellogenin. This lag phase is the time required for assembly and secretion of the conjugated protein.[655]

[649] P. Henkart, S. Humphreys, and T. Humphreys, *Biochemistry*, 1973, **12**, 3045.
[650] C. B. Cauldwell, P. Henkart, and T. Humphreys, *Biochemistry*, 1973, **12**, 3051.
[651] A. R. Njogu, in 'Trypanosomiasis and Leishmaniosis', ed. K. Elliott, M. O'Connor, and G. E. W. Wolstenholme, Elsevier Publishing Co., Amsterdam, 1974, p. 194.
[652] M. Asakawa, *Mem. Fac. Educ., Kumamoto Univ.*, 1972, **21**, 53.
[653] R. Gatti, C. Borrone, X. Trias, and P. Durand, *Lancet*, 1973, No. 2, 1024.
[654] B. G. Kousseff, N. G. Beratis, C. Danesino, and K. Hirschhorn, *Lancet*, 1973, ii, 1387.
[655] A. H. Merry, P. J. Dolphin, K. A. Munday, and M. Akhtar, *Biochem. J.*, 1973, **132**, 459.

6
Enzymes

BY J. F. KENNEDY

Introduction

The long-awaited recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry on the nomenclature of enzymes have now been published.[1] This publication is a revision of the 1964 Recommendations of the International Union of Biochemistry, and was prepared by the Commission on Biochemical Nomenclature with the assistance of an Expert Committee. The following aspects are covered: the classification and nomenclature of enzymes, multiple-enzyme forms, enzyme precursors, units of enzymic activity, symbols of enzyme kinetics, and the nomenclature of electron-transfer proteins. These sections are followed by a list of enzymes giving the recommended trivial name, the systematic name, the reaction catalysed by the enzyme, the Enzyme Commission number and references, and a comprehensive index to the list, which covers not only names in current use, but also disused and incorrect names. The list of enzymes now contains 1770 entries, compared with 874 in the 1964 edition, and includes some 100 carbohydrate hydrolases and lyases, compared with 44 in the 1964 edition.

Under the heading of enzymic catalysis, a book recently published has described the classification and determination of enzymes, the structure of enzymes, the concept of the active site, enzyme kinetics and mechanisms, proteins and co-enzymes, enzyme catalysis, and binding and specificity theories.[2] Another book on the concepts of catalysis and enzyme actions has dealt with chemical energetics and reaction mechanisms, defining and illustrating catalysis, and briefly reviewing the structures and kinetics of enzymes.[3] A discussion of acids and bases proceeded from catalysis by hydronium ions to multiple acid–base catalysis at the active sites of enzymes. The roles of metal ions, electrophiles, and nucleophiles in catalysis of enzymic and non-enzymic systems, and the specificity of enzymic reactions, the control of enzyme systems, enzyme complexes, and artificial enzymes are also covered. A book dealing with the study of enzyme

[1] 'Enzyme Nomenclature', Elsevier, Amsterdam, 1973.
[2] M. L. Bender, 'Mechanisms of Homogenous Catalysis from Protons to Proteins', Wiley, Chichester, 1971.
[3] M. L. Bender and L. J. Brubacher, 'Catalysis and Enzyme Action', McGraw-Hill, New York, 1973.

mechanisms covers the following aspects: enzyme methods, studies of chemical modification, kinetics, mechanisms of catalysis, organic co-enzymes, hydrolytic enzymes, enzymic oxidations, allosterism, and the classification of enzymes.[4]

The application of affinity chromatography, including affinants based on polysaccharides, to the purification, isolation, and preparation of enzymes has been reviewed;[5, 6] applications to the isolation of chemically modified enzymes and to the exploration of enzyme–substrate interactions have also been covered.[5]

On the basis of experiments with two β-galactosidases, it has been suggested that the use of sugar-acid lactones coupled covalently to an inert support may provide a method generally applicable to the purification of lysosomal acid glycohydrolases.[7]

Published versions of papers presented at the Engineering Foundation Conference on Enzyme Engineering have described the isolation, purification, and immobilization of enzymes, and the use of enzymes in reactors.[8] Kinetic aspects of bound and free forms of enzymes were compared.

An article on enzymes of glycoprotein structure has described the types of glycoprotein enzymes (carbohydrate, nucleic acid, and other hydrolases and oxidoreductases), the chemical and physical structure of glycoenzymes (criteria of purity, protein component, carbohydrate–protein linkages, carbohydrate components, and conformation), the biosynthetic pathways (cellular locale, reactions and mechanisms, and regulation of synthesis), and their biological and structural significance.[9]

Within the bounds of data analysis in biochemistry and biophysics, articles have been published on the mathematical background to enzyme kinetics[10] and transient enzyme kinetics (rapid reactions).[11] The kinetics of product inhibition in the ternary-complex mechanism for enzymic reactions involving two substrates have been investigated.[12] Relationships for the effects of product inhibition on steady-state kinetic Dalziel coefficients for enzymes operating by a random-order, ternary-complex mechanism for a two-substrate reaction have been derived; they showed that Dalziel coefficients, in general, are non-linear functions of the concentration of the product used as inhibitor. At low concentrations of product, the coefficient becomes linearly dependent upon the concentration of inhibitor.

[4] E. Zeffren and P. L. Hall, 'The Study of Enzyme Mechanisms', Wiley, Chichester, 1973.
[5] P. Cuatrecasas, *Adv. Enzymol.*, 1972, **36**, 29.
[6] I. A. Cherkasov, *Uspekhi Khim.*, 1972, **41**, 1911.
[7] J. N. Kanfer, G. Petrovich, and R. A. Mumford, *Analyt. Biochem.*, 1973, **55**, 301.
[8] 'Enzyme Engineering', ed. L. B. Wingard, Interscience, New York, 1972.
[9] J. H. Pazur and N. N. Aronson, *Adv. Carbohydrate Chem. Biochem.*, 1973, **27**, 301.
[10] M. E. Magar, 'Data Analysis in Biochemistry and Biophysics', Academic Press, New York, 1972, p. 429.
[11] M. E. Magar, 'Data Analysis in Biochemistry and Biophysics', Academic Press, New York, 1972, p. 442.
[12] G. Pettersson, *Acta Chem. Scand.*, 1972, **26**, 3935.

Product-linear, Dalziel coefficients can be related to velocity constants in the random-order mechanism through diagnostically valuable equations that are of general applicability within the mechanism, and which permit the formulation of useful kinetic criteria. The subject of conformational adaptability in enzymes has been reviewed.[13]

An attempt has been made to predict theoretically and quantitatively the change in action pattern accompanying modification of one of the subsite affinities of a polymer-degrading enzyme.[14] As a model system, the hydrolyses of linear malto-oligosaccharides catalysed by α-amylase Taka-amylase A were chosen, since, in this case, all the subsite affinities have been determined. Calculations showed that a variety of action patterns could result from chemical modification of one of the subsites. A review on a synthetic model for an enzyme has described a model based on a cycloamylose that catalysed some hydrolytic reactions of synthetic esters.[15] The hollow arising from the structure and conformation of the cycloamylose molecule is considered to imitate the classical view of the spatially active site of the enzyme. On the introduction of nickel chelated with pyridinecarboxadoxine as an artificial catalytic group into this site, a highly active hydrolase was formed, the basic macromolecular skeleton being a polysaccharide, in contrast to the protein of natural enzymes.

A collection of articles on practical clinical enzymology has been published in book form and covers methods of enzyme analysis, techniques, and interpretations in chemical–biochemical profiling, as well as enzyme patterns and abnormalities in clinical conditions.[16]

A general method for continuously monitoring the spectrophotometric assay of glycosidases at all values of pH using 4-nitrophenyl glycosides as substrates has been reported.[17] A number of different wavelengths of light may be used for the spectrophotometric measurements, according to the criteria laid down by the user, *e.g.* maximum sensitivity at a selected pH, determination of the pH optimum of the enzyme with a pH-independent, difference-extinction coefficient, or the reduction of background absorbance for kinetic studies at high concentrations of substrate. The method was demonstrated specifically by the development of routine assays for α-galactosidase and β-acetamidodeoxyhexosidase. Application of a computer to automating a recording polarimeter for enzymic assays has been reported.[18] The program was divided into sections dealing with the following: introduction, calculation of the molar conversion factor, incubation time and chart speed, line-voltage calculations, line-reagent

[13] N. Citri, *Adv. Enzymol.*, 1973, **37**, 397.
[14] K. Hiromi, M. Ohnishi, and S. Shibata, *J. Biochem.* (*Japan*), 1973, **74**, 397.
[15] V. Kaláč and K. Babor, *Chem. listy*, 1972, **66**, 1299.
[16] 'Practical Clinical Enzymology: Techniques and Interpretations and Biochemical Profiling', ed. P. L. Wolf, D. Williams, and E. Von der Muehll, Wiley, Chichester, 1973.
[17] J. R. Ford, J. A. Nunley, Y. T. Li, R. P. Chambers, and W. Cohen, *Analyt. Biochem.*, 1973, **54**, 120.
[18] W. T. Lowry, J. R. Vercellotti, and A. S. Carrell, *Carbohydrate Res.*, 1973, **28**, 93.

and reagent-mixture calculations, stock solutions, gradient calibration, assay conditions, protein concentration, sample pattern, results, and computer subroutines. The technique was applied to the reactions of a number of carbohydrate hydrolases, using 4-nitrophenyl glycopyranosides as substrates, and to the gel filtration and disc gel electrophoresis of these enzymes.

Methods for the assay and purification of, and for determining the properties of, glycosidases, glycolipid-specific glycosidases, oligosaccharidases, polysaccharidases, and β-aspartylacetamidodeoxyglucosidase have been described in a series of articles.[19]

The stereochemistry of enzymic reactions catalysed by glycosidases, *endo*-polysaccharidases, *exo*-polysaccharidases, and carbohydrate isomerases has been reviewed.[20] The cleavage of glycosides and disaccharides was shown to occur with retention of the anomeric configuration, whereas the action of *exo*-polysaccharidases proceeded with inversion of the anomeric configuration of the liberated carbohydrate. *endo*-Polysaccharidases have an action pattern in which cleavage occurs with retention of the anomeric configuration. Determination of the anomeric configuration of the products resulting from the action of a carbohydrase has been based on the observation that the sign of the accumulation rate of the P_1-anomer in the process

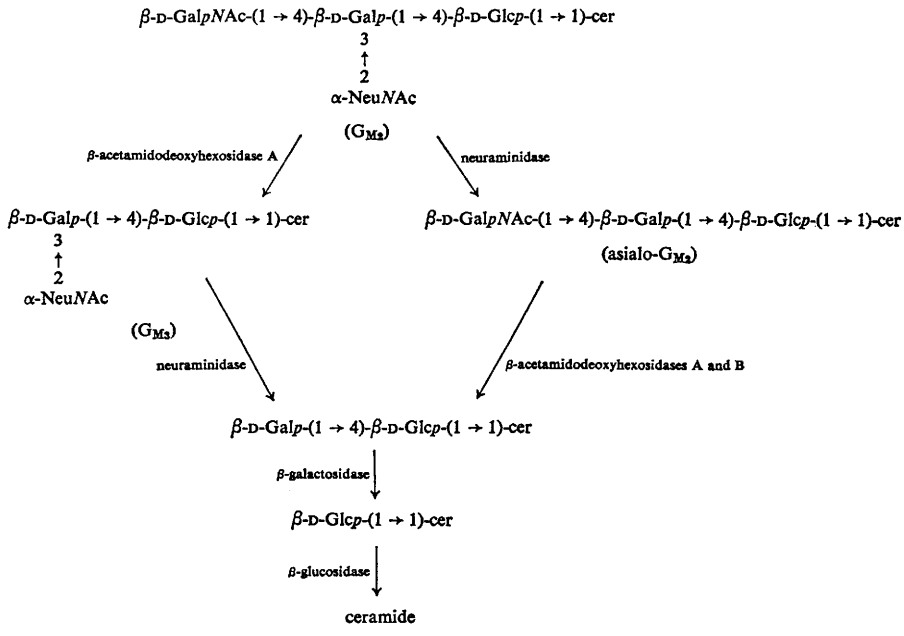
$$\text{substrate} \xrightarrow{\text{enzyme}} \text{anomer } P_1 \rightleftharpoons \text{anomer } P_2$$

can change under certain conditions, whereas that of the P_2-anomer remains positive.[21] This was confirmed by a ^1H n.m.r. study of the kinetics of two stereochemically known enzymic reactions, *viz.* the hydrolysis of amylopectin by barley β-amylase and the hydrolysis of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside by boar-epididymis β-acetamidodeoxyglucosidase. Thus, whilst a direct ^1H n.m.r. procedure has some limitations, kinetic studies of the anomer accumulation using ^1H n.m.r. techniques are generally applicable.

Inhibition of glycosidases by aldonolactones has been comprehensively reviewed.[22] Structural relationships and the specificity and mechanism of the inhibition have been considered in detail. 1-Aminoglycosides have been considered to represent a new class of specific and relatively potent inhibitors of glycosidases.[23] For a number of different glycosidases from various sources, the compounds were found to be specific against enzymes that act upon glycosides corresponding to the glycone of the inhibitor

[19] 'Methods in Enzymology', Vol. XXVIII, ed. S. P. Colowick and N. O. Kaplan, 'Complex Carbohydrates, Part B', ed. V. Ginsburg, Academic Press, New York, 1972, p. 699.
[20] R. Bentley, *Ann. Rev. Biochem.*, 1972, **41**, 953.
[21] I. V. Vikha, V. G. Sakharovsky, V. F. Bystrov, and A. Y. Khorlin, *Carbohydrate Res.*, 1972, **25**, 143.
[22] G. A. Levvy and S. M. Snaith, *Adv. Enzymol.*, 1972, **36**, 151.
[23] H. L. Lai and B. Axelrod, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 463.

β-D-Galp-(1 → 3)-β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc-(2 → ?)-α-NeuNAc
(GT1a)
neuraminidase
β-D-Galp-(1 → 3)-β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc
(GD1a)
neuraminidase
β-D-Galp-(1 → 3)-β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc
(GM1)
β-galactosidase
β-D-Galp-(1 → 3)-β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc
α-NeuNAc-(2 → 8)-α-NeuNAc
(GT1b)
neuraminidase
β-D-Galp-(1 → 3)-β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc-(2 → 8)-α-NeuNAc
(GD1b)
β-galactosidase
β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc-(2 → 8)-α-NeuNAc
neuraminidase
(GD2)


β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc
(GM3)
β-acetamidodeoxyhexosidase A
neuraminidase
β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
3
↑
2
α-NeuNAc
(GM3)
β-D-GalpNAc-(1 → 4)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
(asialo-GM3)
neuraminidase
β-acetamidodeoxyhexosidases A and B
β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-cer
β-galactosidase
β-D-Glcp-(1 → 1)-cer
β-glucosidase
ceramide

Scheme 1

molecule. 1-Glycopyranosylimidazoles have been found to act as inhibitors of glycosidases.[24] The exceptional stability of the glycosyl linkages in these molecules is considered to render them of particular value for studies of glycosidase–inhibitor interactions.

A number of carbohydrate azido-derivatives have been synthesized as potential affinity labels for glycosidases; each contained an azido-group that could be converted into a reactive nitrene by photolysis.[25] The nitrene is considered to be capable of forming a covalent bond with almost any amino-acid residue at the active sites of glycosidases.

A complete scheme for the enzymic hydrolysis of gangliosides by various glycosidases has been reported (see Scheme 1).[26]

The subject of immobilized enzymes has been reviewed under the following headings: carriers, properties of bound enzymes (stability and kinetic patterns), methods of application (stirred tank reactors, columns, *etc.*), and potential applications (industrial and medical uses, *etc.*).[27] A related review has dealt with the covalent attachment of enzymes to water-insoluble, functionalized polymers, the properties of covalently insolubilized enzymes, the intermolecular cross-linking of enzymes with multifunctional reagents, the adsorption of enzymes, the entrapment of enzymes within cross-linked polymers, microencapsulation, containment within semipermeable membrane devices, enzyme reactors, and the applications of immobilized enzymes.[28]

Attention has been drawn to the importance of steady-state, non-linear diffusion equations that describe reactions in constrained solutions of enzymes, and to many applications of constrained enzymes in biology and engineering.[29] Although, as in other types of non-linear differential equations, exact analytical solutions do not exist except in simplified cases, a general procedure has been devised for solving numerically for the substrate-concentration profile and for the effectiveness factor of immobilized enzyme catalysts. Design correlations for enzyme solutions constrained within spherical membranes were included. The use of a unique definition of the Thiele modulus in the charts has permitted the effects of substrate concentration and external, mass-transfer resistances on the overall effectiveness factor of the catalyst particle to be illustrated. On the basis of theoretical treatments of the kinetics of solid-supported enzymes in which diffusion effects are significant, methods have been suggested for analysing experimental results.[30] The procedures can be applied to membranes, spherical particles, and rods. The Michaelis–Menten law

[24] E. J. Bourne, P. Finch, and A. G. Nagpurkar, *Carbohydrate Res.*, 1973, **29**, 492.
[25] E. Saman, M. Claeyssens, H. Kersters-Hilderson, and C. K. de Bruyne, *Carbohydrate Res.*, 1973, **30**, 207.
[26] J. F. Tallman and R. O. Brady, *Biochim. Biophys. Acta*, 1973, **293**, 434.
[27] K. L. Smiley and G. W. Strandberg, *Adv. Appl. Microbiol.*, 1972, **15**, 13.
[28] O. R. Zaborsky, 'Immobilized Enzymes', C.R.C. Press, Cleveland, 1973.
[29] D. J. Fink, T.-Y. Na, and J. S. Schultz, *Biotechnol. and Bioeng.*, 1973, **15**, 879.
[30] T. Kobayashi, *Biochim. Biophys. Acta*, 1973, **302**, 1.

applies to such systems, but the kinetic parameter K_m is only an apparent one, since it is influenced by partitioning and diffusional effects. The methods suggested allow the true kinetic parameters, relating to the behaviour of the enzyme within the support, to be derived from the experimental results. One method is applicable when the experimental results relate to various concentrations of substrate at either a constant membrane thickness or particle diameter. Other methods are useful when data exist for various membrane thicknesses or particle diameters at a constant concentration of substrate. The influence of diffusion on the apparent thermal stability of a reversibly or an irreversibly denaturable enzyme has been examined theoretically for the enzyme in uniform distribution in a porous solid.[31] If the overall reaction rate is influenced by diffusion through the catalyst, the insolubilized catalyst was shown to yield an apparently more thermally stable enzyme, even though the maximal velocity and the K_m value are the same for the free and insolubilized forms of the enzyme. Brief attention was given to the experimental conditions necessary to demonstrate conclusively whether or not insolubilization affects the thermal stability of the enzyme.

Acetamidodeoxygalactosidases, Acetamidodeoxyglucosidases, and Acetamidodeoxyhexosidases

A general method for continuously monitoring the spectrophotometric assay of glycosidases at all values of pH using 4-nitrophenyl glycosides as substrates has been reported; the method was exemplified in the case of β-acetamidodeoxyhexosidase activity using the enzyme from Jack-bean meal at pH 5 and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside as the substrate.[17] A number of different wavelengths of light may be used for the assay, according to the requirements of the operator, *e.g.* maximum sensitivity at a selected pH, determination of the pH optimum of the enzyme with a pH-independent, difference-extinction coefficient, or the reduction of background absorbance for kinetic studies at high concentrations of substrate. Investigation of the u.v. absorption spectra of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 4-methylumbelliferone showed them to differ significantly at 350 nm, and this finding was applied to the enzymic hydrolysis of a number of 4-methylumbelliferyl glycosides using a continuous spectrophotometric assay.[32] With the aid of this automated technique, selected kinetic properties (K_i, K_m, and V_{max}) of partially purified β-acetamidodeoxyhexosidases A and B from human liver and of crystalline β-acetamidodeoxyhexosidase from Jack-bean meal were determined and found to be in close agreement with known data obtained by conventional single-point assays. The technique is fast and accurate, and permits instantaneous measurement of

[31] D. F. Ollis, *Biotechnol. and Bioeng.*, 1972, **14**, 871.
[32] A. L. Rosenthal and A. Saifer, *Analyt. Biochem.*, 1973, **55**, 85.

the kinetic properties of certain enzymes implicated in a number of genetic disorders.

β-Acetamidodeoxyhexosidase activities have been determined in tissues from a variety of clinical conditions in children.[33]

A new, reproducible method for the extraction and assay of lysosomal β-acetamidodeoxyglucosidase from human skin has been reported.[34] Biopsy specimens from normal skin were frozen, sectioned on a cryostat, and homogenized; the enzyme was demonstrated to be stable under the conditions used and its kinetic characteristics were evaluated. The specific activity of the enzyme was found to be eight- to ten-fold higher than that previously reported.

β-Acetamidodeoxyhexosidases A and B have been extracted from human-skin fibroblasts and separated by ion-exchange chromatography.[35] The initial extract and two separated components were utilized in establishing the K_m and V_{max} values for four substrates: 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galactopyranoside, 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside, 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside, and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. The K_m values obtained for the D-*gluco*-compounds are essentially similar. Use of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galactopyranoside yielded similar K_m values for the three enzyme preparations, but differences were encountered when 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside was used as the substrate. Several batches of commercial serum albumin were found to contain appreciable β-acetamidodeoxyhexosidase activity. β-Acetamidodeoxyhexosidase A has been purified from crude extracts of human-skin fibroblasts or liver by affinity chromatography.[36] The affinity-chromatography column was prepared by the attachment to a Sepharose derivative of a glycopeptide obtained from bovine nasal septum by sequential treatments with trypsin, chymotrypsin, methanolic hydrogen chloride, hyaluronidase, and β-glucuronidase. The column selectively retarded the passage of the β-acetamidodeoxyhexosidase A activity of the extracts, which could be eluted subsequently with Triton X-100 in citrate–phosphate buffer (pH 4.4). Skin fibroblasts cultured from patients with Tay–Sachs disease, which are characterized by a deficiency in β-acetamidodeoxyglucosidase, were able to take up β-acetamidodeoxyglucosidase derived from normal lysosomes, whereas the use of β-acetamidodeoxyglucosidase from the culture medium of skin fibroblasts from patients with I-cell disease exhibited a reduced ability to be taken up.[37] The levels of β-acetamidodeoxyhexosidase activity have been measured in homogenates of the fibroblasts from

[33] M. Sugita, J. T. Dulaney, and H. W. Moser, *Science*, 1972, **178**, 1100.
[34] J. C. Steigerwald and B. A. Bartholomew, *Biochim. Biophys. Acta*, 1973, **321**, 256.
[35] J. N. Kanfer and C. Spielvogel, *Biochim. Biophys. Acta*, 1973, **293**, 203.
[36] G. Dawson, R. L. Propper, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1102.
[37] S. Hickman and E. F. Neufeld, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 992.

normal individuals, from patients affected with disorders associated with a deficiency of α-L-iduronidase (Hurler syndrome, Scheie syndrome, and a Hurler–Scheie compound condition), and from the parents of these patients.[38] The levels of activity were compared with those measured for other glycosidases in the same homogenates.

β-Acetamidodeoxyhexosidases A and B have been partially purified from extracts of normal human liver by ion-exchange chromatography.[39] β-Acetamidodeoxyhexosidase B was similarly purified from the livers of cases of Tay–Sachs disease. The two enzymes from normals showed similar abilities to catalyse the hydrolysis of synthetic 2-acetamido-2-deoxy-β-D-glucopyranosides and 2-[1-^{14}C]acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide, whereas the enzyme from Tay–Sachs disease possessed slightly higher K_m values towards the same substrates. Both the normal enzymes hydrolysed globoside (B having a lower K_m value than A), whereas neither enzyme hydrolysed ganglioside unless the *N*-acetylneuraminic acid units had been removed previously. However, both globoside and ganglioside were excellent competitors of the hydrolysis of the triglycosylceramide. The results were discussed with respect to the accumulation of these glycolipids in ganglioside-storage diseases. Since β-acetamidodeoxyhexosidase A of human tissues can be converted into a form resembling, but not necessarily identical to, β-acetamidodeoxyhexosidase B by enzymic removal of sialic acid residues, it has been proposed that the enzymes consist of at least two different subunits: one of these, being common to all forms of the enzyme, carries the active site for hydrolysis and is largely responsible for the observed antigenicity, whereas the other is a specific subunit characteristic of β-acetamidodeoxyhexosidase A and confers on the active site the specific ability to hydrolyse ganglioside G_{M2} {2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-*O*-[α-*N*-acetylneuraminosyl-(2 → 3)]-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide}.

A protein of low molecular weight having several of the properties expected for the proposed subunit has now been detected and partially characterized in livers from normals and cases of Sandhoff's and Tay–Sachs diseases.[40] From gel-filtration studies, it was found that cross-reactivity occurred in samples containing active β-acetamidodeoxyhexosidase (molecular weight 1.3 × 10^5) and also in a component of lower molecular weight (2.0—2.5 × 10^4). Sandhoff and Tay–Sachs tissues showed not only macromolecular, cross-reacting material corresponding in size to the complete enzyme, but, in addition, considerable amounts of the species of lower molecular weight, which was entirely devoid of hydrolytic activity towards the synthetic substrates used. The macromolecular, cross-reacting material of Sandhoff tissues co-chromatographed with the low, but

[38] C. W. Hall and E. F. Neufeld, *Arch. Biochem. Biophys.*, 1973, **158**, 817.
[39] D. A. Wenger, S. Okada, and J. S. O'Brien, *Arch. Biochem. Biophys.*, 1972, **153**, 116.
[40] D. Robinson, M. Carroll, and J. L. Stirling, *Nature*, 1973, **243**, 415.

measurable, amount of β-acetamidodeoxyhexosidase activity present in such cases, whereas, in Tay–Sachs tissues, it could not be distinguished from a considerable amount of residual β-acetamidodeoxyhexosidase. Antisera raised to a partially purified preparation of β-acetamidodeoxyhexosidase from human liver and to β-acetamidodeoxyhexosidase isoenzymes A and B precipitated the enzyme in an enzymically active form, which could be located on immunodiffusion and immunoelectrophoretic gels using a histochemical substrate.[41] Antisera to the purified isoenzymes reacted with β-acetamidodeoxyhexosidases from human liver, kidney, brain, and spleen, but they did not cross-react with β-glucosidase, β-galactosidase, β-mannosidase, β-xylosidase, aryl sulphatase, and acid phosphatase from human liver. The isoenzymes A and B of β-acetamidodeoxyhexosidase were shown to be immunologically identical, and the β-acetamidodeoxyhexosidases from the livers of patients with three types of G_{M_2}-gangliosidosis possessed immunological properties similar to one another. No evidence for cross-reacting material in enzyme-deficient states could be found.

Serum levels of β-acetamidodeoxyhexosidase in cases of cystic fibrosis have been measured for homozygotes and heterozygotes.[42] Metachromasia-negative donors generally exhibited higher total levels of enzyme compared with normals, but the distribution of A and B isoenzymes remained normal. In contrast, metachromasia-positive donors exhibited total levels of the enzyme lower than normal, although the percentage of the A and B isoenzymes was high and low, respectively, a situation that is the reverse of the carrier profile for Tay–Sachs disease.

The activities of the β-acetamidodeoxyhexosidases from human cerebrospinal fluid on 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside and -galactopyranoside have been investigated.[43] The pH optimum for enzymic activity was found to be 4.8 for both substrates, and the stabilities of the activities at 50 °C followed one another when either substrate was used. Assessment of the β-acetamidodeoxyhexosidase A and B activities by heat denaturation was reported.

Measurement of the β-acetamidodeoxyhexosidase A and B activities by electrophoresis and by enzyme assay using 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside as substrate has been applied to the *in utero* diagnosis of Sandhoff's disease.[44] The utility of the method is based on the finding that there is a deficiency of β-acetamidodeoxyhexosidase in such cases. Deficiencies of the enzyme were also demonstrated in the plasma and various tissues of the aborted foetus. These findings indicate that maternal β-acetamidodeoxyhexosidases do not cross the foetal–placental barrier.

[41] M. Carroll and D. Robinson, *Biochem. J.*, 1973, **133**, 91.
[42] J. H. Conover, E. J. Conod, and K. Hirschhorn, *Lancet*, 1973, **I**, 1122.
[43] J. L. Viallard, C. Motta, and G. Dastugue, *Compt. rend. Soc. Biol.*, 1973, **166**, 1704.
[44] R. J. Desnick, W. Krivit, and H. L. Sharp, *Biochem. Biophys. Res. Comm.*, 1973, **51**, 20.

Analysis of human urinary β-acetamidodeoxyhexosidase by chromatography on DEAE-cellulose revealed the presence of both A and B isoenzymes, together with a new, minor component designated β-acetamidodeoxyhexosidase M.[45] The M-form, found only in the urine of males, exhibited similar enzymic properties to that of the A-form. The patterns of β-acetamidodeoxyhexosidase isoenzymes for normal individuals and Tay–Sachs heterozygotes were qualitatively similar, but β-acetamidodeoxyhexosidase A was shown to be absent from the urine of a patient with Tay–Sachs disease.

Determination of the anomeric configuration of products resulting from the action of a carbohydrase has been based on the observation that the sign of the accumulation rate of the P_1-anomer in the process

$$\text{substrate} \xrightarrow{\text{enzyme}} \text{anomer } P_1 \rightleftharpoons \text{anomer } P_2$$

could change under certain conditions, whereas the accumulation rate of the P_2-anomer remains positive.[21] This have been confirmed by a ^1H n.m.r. kinetic study of the hydrolysis of a 2-acetamido-2-deoxy-β-D-glucopyranoside by boar-epididymis β-acetamidodeoxyglucosidase as an enzymic reaction of known stereochemistry. 2-Acetamido-2-deoxy-D-glucono-1,5-lactone has been found to undergo isomerization to 2-acetamido-2-deoxy-D-glucono-1,4-lactone when exposed to β-acetamidodeoxyhexosidase from boar epididymis.[46] This transformation was explained in terms of an interaction between the catalytic groups of the enzyme and the carbonyl function of the 1,5-lactone.

The activity and intracellular distribution of β-acetamidodeoxyglucosidase in rabbit liver have been examined.[47] Since the steroid oestrogen has been found endogenous to the rabbit, both oestrogen and 4-nitrophenyl β-glycosides of 2-acetamido-2-deoxy-D-glucopyranose were used as substrates, but no difference was found in the intracellular distribution of β-acetamidodeoxyglucosidase activity towards these substrates. The β-acetamidodeoxyglucosidase was shown to be concentrated in the lysosomes and to exhibit the latency typical of lysosomal acid hydrolases.

The pH optima of β-acetamidodeoxy-galactosidase and -glucosidase in both normal and scrapie-affected mouse brains have been determined, and all were found to coincide at pH 4.5.[48] The two enzyme activities were found to be significantly increased above normal in the affected brain, and elevations of these activities also occurred before the mice exhibited symptoms of the disease.

The development of β-acetamidodeoxyglucosidase as a lysosomal hydrolase has been examined by analysis of bulk-isolated nerve-cell

[45] E. E. Grebner and J. Tucker, *Biochim. Biophys. Acta*, 1973, **321**, 228.
[46] A. Y. Khorlin, G. V. Vikha, M. L. Shulman, V. V. Kolesnikov, and E. D. Kaverzneva, *Biokhimiya*, 1973, **38**, 1095.
[47] J. D. Mellor, D. S. Layne, J. E. Irvin, and A. Mellors, *Canad. J. Biochem.*, 1973, **51**, 1292.
[48] G. C. Millson and L. Bountiff, *J. Neurochem.*, 1973, **20**, 541.

bodies from rat cerebral cortex.[49] A method for solubilization of upwards of 50% of the activity present was established. Centrifugation of a preparation of soluble neuronal β-acetamidodeoxyglucosidase in linear density gradients of sucrose resulted in complete resolution of the activity into components of high and low density, irrespective of the age of the animal from which the sample was taken. It was found routinely that the ratio of the amounts of components of low and high density is greater than unity for cerebral cortex, whereas ratios of less than unity were noted for cerebellar neurons. However, the qualitative and quantitative gradient-profiles of β-acetamidodeoxyglucosidase were shown to be highly sensitive to the pH of the medium. The sensitivities of the enzymes to heat were also studied. The molecular weights of the high- and low-density components of β-acetamidodeoxyglucosidase of the cerebellar neurons were estimated to be approximately 1.58×10^5. The high-density component could be converted into the low-density form in acidic media, and the conversion did not require the presence of exogenous neuraminidase. β-Acetamidodeoxygalactosidase activity has been studied in bulk-separated neuronal and neuropil fractions from rat cerebral cortex and in subcellular fractions derived therefrom.[50] Although the enzyme activity evinced some latency properties, its pattern of subcellular distribution is broader than that observed with other acid hydrolases. By contrast with nine other acid hydrolases, the β-acetamidodeoxygalactosidase was demonstrated to be more active in neuropil than in neuronal fractions, and this feature was preserved in lysosomal subfractions derived from isolated cell fractions.

In a study of the lysosomal glycosidases of rat liver, the subcellular distribution of the β-acetamidodeoxyglucosidase activity was investigated.[51] A study of the principles of affinity chromatography by enzyme–substrate interactions has been applied to the purification of certain rat-liver glycosidases.[52] However, resolution of mixtures of the glycosidases was difficult since, for example, mixtures of β-acetamidodeoxyglucosidase and β-glucuronidase possessed similar affinities towards the binding glycone moieties. The β-acetamidodeoxyglucosidase was absorbed by matrices to which either 4-aminophenyl β-D-glucopyranuronoside or 4-aminophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside had been attached *via* the free amino-groups. The enzyme could be eluted from the various affinants by ionic solutions or solutions of the substrate or inhibitor. β-Acetamidodeoxyglucosidase was separable from β-glucuronidase by means of a matrix to which 4-aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside had been attached and by elution with a stepwise, rather than a linear, concentration of sodium chloride solutions.

[49] O. Z. Sellinger, J. C. Santiago, M. A. Sands, and B. Furin-Sloat, *Biochim. Biophys. Acta*, 1973, **315**, 128.
[50] A. K. Sinha and S. P. R. Rose, *J. Neurochem.*, 1973, **20**, 39.
[51] J. H. LaBadie and N. N. Aronson, *Biochim. Biophys. Acta*, 1973, **321**, 603.
[52] E. Junowicz and J. E. Paris, *Biochim. Biophys. Acta*, 1973, **321**, 234.

A plasma-membrane fraction isolated from the 4-dimethylaminoazobenzene-induced rat hepatoma D23 has been investigated using an immunodiffusion method combined with a staining reaction for β-acetamidodeoxyglucosidase.[53] The enzymically active antigen was also detected, with varying degrees of distribution, in tumour microsomes.

Glycosidase activities have been determined in different parts of the male reproductive tract of the giant octopus (*Octopus dofleini martini*).[54] The spermatophore and the prostate were shown to be major sources of these enzymes, the β-acetamidodeoxyglucosidase activity being concentrated in the cement liquid.

Acetamidodeoxyglucosidases of an extract of the digestive glands of the limpet (*Patella vulgata*) have been purified by ion-exchange chromatography and gel filtration.[55] Both α- and β-acetamidodeoxyglucosidase activities were detected when 4-nitrophenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides were used as substrates. The two activities appeared together in all fractions from the purification procedure and when the enzyme fraction was subjected to isoelectric focusing. The results suggested that a single species of protein is responsible for the two enzyme activities, and this is the first report of such a situation. Further studies showed that a β-acetamidodeoxyglucosidase could be isolated from the digestive glands of limpets in addition to the αβ-acetamidodeoxyglucosidase.[56] Both enzyme species produced two types of subunit on treatment with urea and fractionation by gel filtration. Both enzyme molecules gave rise to subunits having molecular weights of 8.2×10^4 (A) and 5.4×10^4 (B). Since the molecular weights for the αβ- and β-enzymes were estimated to be 2.17×10^5 and 1.36×10^5, respectively, it is considered that the two enzymes consist of two A- and one B-, and one A- and one B-type subunits, respectively. The amino-acid and carbohydrate contents of the two A-type subunits in the αβ-enzyme were shown to be almost exactly the same as those of the single A-type subunit of the β-enzyme, and included hexose (47) and 2-amino-2-deoxyglucose (7 residues per mole). Similarly, analyses of the amino-acids and carbohydrates of the B-type subunits were identical for the two enzymes. The results suggested that the corresponding subunits of the two enzymes may be identical, and this conclusion was verified by immunodiffusion experiments. The results provide the first direct evidence for the existence of subunits of acetamidodeoxyglucosidases.

Purification of the β-acetamidodeoxyglucosidase from germinating seeds of *Dolichos biflorus* by means of ion-exchange chromatography has been described.[57] The enzyme exhibited optimum activity at pH 4.5—5.5, and its storage properties were also investigated.

[53] F. Blomberg and M. Raftell, *Biochim. Biophys. Acta*, 1973, **291**, 431.
[54] T. Mann, A. Karagiannidis, and A. W. Martin, *Comp. Biochem. Physiol.*, 1973, **44A**, 1377.
[55] J. V. Bannister and P. J. R. Phizackerley, *F.E.B.S. Letters*, 1973, **29**, 313.
[56] J. V. Bannister and P. J. R. Phizackerley, *F.E.B.S. Letters*, 1973, **34**, 120.
[57] D. Meyer and R. Bourrillon, *Biochimie*, 1973, **55**, 5.

β-Acetamidodeoxyglucosidase from Jack-bean meal has been purified by fractional precipitation with ammonium sulphate, followed by ion-exchange chromatography and gel filtration; ion-exchange chromatography separated the β-acetamidodeoxyglucosidase from an α-mannosidase also present in the original extract.[58]

In order to investigate the specificity of β-acetamidodeoxyglucosidase from Takadiastase (an enzyme preparation containing α-amylase from *Aspergillus oryzae*) towards *N*-acyl groups, the activity of the enzyme towards 2-acylamino-2-deoxy-β-D-glucopyranosides containing formyl, propionyl, n-butyryl, isobutyryl, and benzoyl groups as *N*-substituents was investigated.[59] Substrates other than the *N*-benzoyl derivative were hydrolysed by the enzyme, demonstrating that the specificity is not restricted to the *N*-acetyl group, although the latter was found to be the most favourable group for enzymic action. The specificity is considered to be controlled by a steric factor(s) associated with the *N*-substituent, although other factors such as hydrophobic and electronic effects are also presumed to be involved. Further studies of the specificity of the β-acetamidodeoxyglucosidase towards *N*-acyl groups have been carried out using 4-nitrophenyl 2-acylamino-2-deoxy-β-D-glucopyranosides with monofluoro-, monochloro-, monobromo-, difluoro-, dichloro-, and trifluoro-acetyl groups as *N*-substituents.[60] All the substrate analogues, with the exception of the dichloro-compound, were hydrolysed by the β-acetamidodeoxyglucosidase. Comparison of the rate of hydrolysis of each substrate analogue with that of the *N*-acetyl compound led to the conclusion that monohalogen substitution of the *N*-acetyl group, or even di- or tri-substitution in the case of fluorine, is permissible, and that the specificity is largely controlled by steric factors related to the *N*-substituent of the substrate.

Takadiastase β-acetamidodeoxyglucosidase has been shown to hydrolyse [2-acetamido-2-deoxy-β-D-glucopyranoside-(1 → 4)]$_4$-2-acetamido-2-deoxy-D-glucitol from the non-reducing end.[61] A study of the rates of hydrolysis of chitin oligosaccharides and reduced chitin oligosaccharides revealed that the active site of the enzyme consists of two subsites. Values for the free energy of binding of phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and of the corresponding 3- (*etc.*) deoxy-analogues were calculated from the K_m values. The contribution to the overall binding by each hydroxy-group, defined as the partial free energy of binding, was estimated from differences between the total binding energies for each analogue and that of phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside. Contributions to the binding made by acetamido- and phenoxy-groups were derived from the inhibitor constants for phenyl β-D-glucopyranoside and 2-acetamido-1,5-anhydro-2-

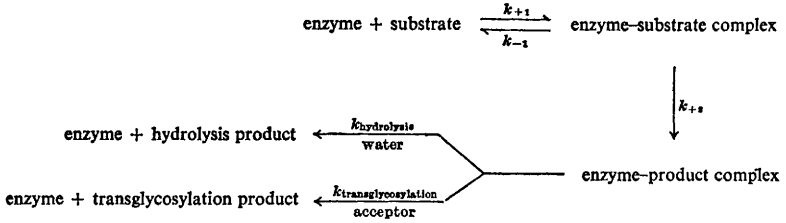
[58] B. J. Catley, *Arch. Biochem. Biophys.*, 1973, **159**, 214.
[59] K. Yamamoto, *J. Biochem.* (*Japan*), 1973, **73**, 631.
[60] K. Yamamoto, *J. Biochem.* (*Japan*), 1973, **73**, 749.
[61] T. Mega, T. Ikenaka, H. Arita, K. Fukukawa, and Y. Matsushima, *J. Biochem.* (*Japan*), 1973, **73**, 55.

deoxy-D-glucitol, respectively. The results indicated the existence of four hydrogen bonds between phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and β-acetamidodeoxyglucosidase, two involving the acetamido-group and two involving the hydroxylic hydrogens at C-3 and C-4. The sum of the partial free energies of binding was found to be almost equal to the total free energy of binding of phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside.

The β-acetamidodeoxyglucosidase from *A. oryzae* Takadiastase has been shown to possess a strong transglycosylation activity; di- and trisaccharides were produced by the transfer reaction when the substrate is present in high concentration.[62] Structural identification of the two disaccharides produced when phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was used as substrate showed that the enzyme transferred the 2-acetamido-2-deoxy-D-glucosyl residue from the substrate to the hydroxy-group at either C-4 or C-6 of another substrate molecule, the respective products being formed in a molar ratio of 13 : 1. When phenyl 2-acetamido-2-deoxy-β-D-galactopyranoside was used as substrate, the transfer reaction hardly occurred, whereas phenyl 2-acetamido-2-deoxy-3- and -6-*O*-methyl-β-D-glucopyranosides both acted as substrates in the transfer reaction. Since the K_m value for each of the latter two substrates is different from that for phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside, it was argued that the lifetimes of the corresponding enzyme–product complexes are different. Thus, it was considered likely that the yield of transfer product does not depend on the lifetime of the enzyme–product complex, but on the ease of combination of the acceptor with the acceptor site on the enzyme–product complex. This view is consistent with the observed lack of effect of a change in the pH of the environment. Since the ratio of formation of (1 → 4)- to (1 → 6)-linkages was less for the 3-*O*-methylated substrate than for the normal substrate, it was also concluded that methylation at C-3 decreases the acceptor ability of the hydroxy-group at C-4. Further studies showed that a number of sugars could act as acceptors for the 2-acetamido-2-deoxy-D-glucosyl residue in the transglycosylation reaction catalysed by *A. oryzae* β-acetamidodeoxyglucosidase.[63] In order to define the nature of the active site of the enzyme, the efficiencies of various molecules as acceptors were examined and the following order was established: 2-acetamido-2-deoxy-D-glucose > 2-deoxy-2-formamido-D-glucose > D-glucose and D-galactose > 2-deoxy-D-*arabino*-hexose > D-mannose. The order among various alcohols tested as acceptors was: primary alcohols > the corresponding secondary alcohols > pentan-1-ol > butan-1-ol > propan-1-ol > ethanol > methanol and glycerol > erythritol > xylitol > D-glucitol > 2-acetamido-2-deoxy-D-glucitol. Tertiary alcohols did not act as acceptor molecules. The results suggest that the structure of the hexose at C-3 is important in the interaction of the acceptor and the acceptor site of the enzyme, that an alkyl chain easily enters the acceptor

[62] T. Mega, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 1197.
[63] T. Mega, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 1391.

site, and that the ability of polyalcohols to act as acceptors decreases with increasing chain-length. The three-step mechanism shown in the equation (Scheme 2) was postulated for the transglycosylation reaction, and the



Scheme 2

ratio $k_{transglycosylation}/k_{hydrolysis}$, defined as the transfer ratio, was determined for several acceptors.

Bacillus subtilis has been shown to produce a β-acetamidodeoxyglucosidase in both simple and complex culture media during the late logarithmic phase of growth.[64] Although the enzyme is partially associated with the organism, it does not accumulate within the organism before release. Addition of 2-acetamido-2-deoxy-D-glucosides to the medium caused an increase in the differential rate of enzyme synthesis, and, in general, synthesis of the enzyme appeared to be regulated by a balance of inductive and repressive effects. Control of the production of β-acetamidoglucosidase by *B. subtilis* growing on a chemically defined medium has been studied; production of the enzyme was repressed during exponential growth by carbon sources that enter the glycolytic pathway above the level of phosphoenol pyruvate.[65] When exponential growth ceased, as a consequence of a fall in nitrogen concentration of the medium to a low level, the enzyme was formed, and its amount could be increased by the addition of fragments of the bacterial cell wall as inducer. These results and those of de-repression studies suggested the existence of a strong link, at the level of the tricarboxylic-acid cycle, between the control of phosphoenolpyruvate carboxylase and that of the de-repression of β-acetamidodeoxyglucosidase and sporulation. The *B. subtilis* enzyme was shown to possess a pH optimum of pH 5.9, a p*I* of 3.8, and a molecular weight of 7.5×10^4.[66] Studies on its ability to attack a wide range of synthetic and natural substrates, and kinetic data for the hydrolyses of a number of these substrates, were reported. The K_m and V_{max} values for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (bacterial cell-wall disaccharide) were 0.15 and

[64] J. M. Ortiz, *J. Gen. Microbiol.*, 1973, **77**, 331.
[65] S. J. Brewer and R. C. W. Berkeley, *Biochem. J.*, 1973, **134**, 271.
[66] R. C. W. Berkeley, S. J. Brewer, J. M. Ortiz, and J. B. Gillespie, *Biochim. Biophys. Acta*, 1973, **309**, 157.

0.018 mmol l^{-1}, and 14.50 and 32.57 μmol min^{-1} mg^{-1}, respectively. The rates of hydrolysis of chitin oligosaccharides decreased with increasing molecular size, but neither 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose nor 2-acetamido-2-deoxy-β-D-galactopyranosides were attacked by the enzyme. From these results, and those of inhibition studies, it was concluded that the enzyme is entirely specific for substrates with non-reducing, terminal 2-acetamido-2-deoxy-D-glucopyranosyl residues.

Evidence has been presented for the occurrence of an *endo*-α-acetamidodeoxygalactosidase in culture filtrates of *Clostridium welchii* (*C. perfringens*).[67] The action of the enzyme on porcine submaxillary glycoprotein resulted in the release of a disaccharide, which was characterized as 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose, and the possibility of the disaccharide arising from a transglycosylation reaction was discounted. The action of the enzyme on several glycoproteins and blood-group substances from various sources also resulted in the production of the same disaccharide. Attention was drawn to the potential utility of the enzyme in structural studies of the carbohydrate moieties of macromolecules.

Purification of the β-acetamidodeoxyhexosidase from the spent growth medium of *Dictyostelium discoideum*, by way of gel filtration and fractionation on hydroxyapatite, gave a form that was electrophoretically homogeneous.[68] The β-acetamidodeoxyglucosidase and β-acetamidodeoxygalactosidase activities appeared to be associated with a single, proteinaceous species. The pH optimum (5.1), pH stability, temperature stability, kinetic properties, and substrate specificity of the enzyme were examined. Reaction of the enzyme with concanavalin A produced strong precipitation bands on Ouchterlony plates, and the precipitate reacted with the corresponding histochemical and fluorogenic substrates. It was concluded that the enzyme is a glycoprotein containing α-linked D-mannose and, possibly, D-glucose and 2-acetamido-2-deoxy-D-glucose residues.

Arabinosidases

One (pH optimum 5.5) of the two β-galactosidases found in a liver extract of the marine gastropod *Turbo cornutus* has been purified by means of fractional precipitation, heat and pH-variation treatments, ion-exchange chromatography, and gel filtration. The enzyme was shown to possess α-L-arabinofuranosidase, β-D-fucosidase, β-galactosidase, and β-glucosidase activities in the ratio 1 : 13 : 5 : 5, using 4-nitrophenyl glycosides as substrates.[69] It is regarded as a glycosidase that hydrolyses glycosidic bonds in substrates having the hydroxy-groups arranged as at C-1, C-2, and C-3 of β-D-galactopyranose.

[67] C. C. Huang and D. Aminoff, *J. Biol. Chem.*, 1972, **247**, 6737.
[68] D. Every and J. M. Ashworth, *Biochem. J.*, 1973, **133**, 37.
[69] M. Yamada, K. Ikeda, and F. Egami, *J. Biochem.* (*Japan*), 1973, **73**, 69.

The inhibitory action of synthetic *C*-glycosides on the α-L-arabinofuranosidase of sweet almond emulsin has been investigated.[70] The inhibition was shown to be non-competitive for 1-α-L-arabinopyranosyl-, 1-β-D-galactopyranosyl-, 1-β-D-glucopyranosyl-, and 1-β-D-xylopyranosyl-4-nitrobenzene.

A new enzyme, a β-L-arabinosidase, has been detected in dormant seeds of *Cajanus indicus* using 4-nitrophenyl β-L-arabinopyranoside as substrate.[71] The enzyme activity, isolated by means of gel filtration, was shown to differ from that of α-D-galactosidase, α-D-fucosidase, and β-D-galactosidase, since the preparation was inactive against the 4-nitrophenyl glycosides of α- and β-D-galactopyranose, α-D-fucopyranose, and β-D-galactopyranose. As purified by gel filtration, the enzyme (molecular weight 2.59×10^4) behaved as a single component on polyacrylamide gel electrophoresis; it exhibited a broad pH optimum (2.5—4.5) and was stable over the pH range 2.0—6.5.

β-Fructofuranosidases

A new, direct, spectrophotometric method for measuring the kinetics of β-fructofuranosidase has been reported.[72] The method depends on the stoicheiometric release of D-glucose from sucrose by the enzyme, and utilizes absorption peaks in the far-u.v. spectra of D-fructose (188.5 and 278 nm) and D-glucose (186 nm). Buffers were found to exert a bathochromic shift and to have a hypochromic effect. Using a wavelength of 207 nm, assay of β-fructofuranosidase in acetate buffer gave a K_m value of 12.3 mmol l^{-1} for sucrose. This result is in good agreement with those obtained either by polarimetry or by chemical assay of released reducing groups (14.6 and 12.3 mmol l^{-1} for sucrose, respectively). The advantages claimed for the method are simplicity, and the ability to measure initial reaction rates and to follow continuously the course of the reaction.

The β-fructofuranosidase activities of the small intestines of humans and of non-human primates (Lorisidae, Hapalidae, and Cebidae) have been determined.[73] Black and red tamarins (*Tamarinus nigricollis*) were found to possess the highest small-intestinal β-fructofuranosidase activity.

The β-fructofuranosidase activity of an extract from the leaves of winter wheat (*Triticum aestivum*) has been separated into three fractions (molecular weights 10^7, 10^6, and $1.2—1.3 \times 10^4$, respectively) by gel filtration.[74] Isoelectric focusing revealed that a few of the β-fructofuranosidase molecules are basic proteins (p*I* values 8.0—8.7), whereas most were acidic (p*I* values 3.2—5.0). The enzyme molecules in the group of highest molecular weight were a mixture of basic (p*I* 8.7) and acidic (p*I* values 3.5—4.8) proteins.

[70] I. A. Zhdanov and R. M. Kessler, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 607.
[71] P. M. Dey, *Biochim. Biophys. Acta*, 1973, **302**, 393.
[72] M. L. Sharma and E. Newbrun, *Carbohydrate Res.*, 1973, **29**, 165.
[73] J. D. Welsh and A. W. Walker, *Comp. Biochem. Physiol.*, 1973, **46A**, 549.
[74] D. W. A. Roberts, *Biochim. Biophys. Acta*, 1973, **321**, 220.

Most of the enzyme proteins in the fraction of medium molecular weight possessed p*I* values of 4.2, 4.3, or 4.8, while the smallest enzyme molecules generally possessed a p*I* of 8.5. The proportions of β-fructofuranosidases in the fractions varied according to the conditions under which the plants were grown.

Soluble and insoluble β-fructofuranosidases have been found in dormant pollen of *Haemanthus albiflos*.[75] The enzymes possessed pH optima of 5.7 and 5.5, respectively, and, at their pH optima, the activity of the soluble enzyme was more than three times higher than that of the insoluble enzyme. After germination of the pollen for two hours, the pH optimum of the insoluble enzyme had increased to pH 6.0 and the activity had increased two-fold, whereas the activity of the soluble β-fructofuranosidase had decreased by 26% under these conditions.

Two inducible enzymes, β-fructofuranosidase and levansucrase, have been reported to be responsible for the saccharolytic activity in *Bacillus subtilis*.[76]

The surface distribution of β-fructofuranosidase on growing cells of *Saccharomyces* sp. has been investigated.[77] An immunoglobulin fraction from antiserum to the enzyme was labelled with fluorescein isothiocyanate, and, with the aid of these antibodies, it was found that most of the β-fructofuranosidase newly formed at the surface of the cell surrounds the developing bud. The β-fructofuranosidase and inulinase functions of the active site of an enzyme from *S. fragilis* have been investigated.[78]

β-Fructofuranosidase has been immobilized by covalent attachment to a polydiazo-type of ion-exchange resin,[79] by cross-linking on a membrane with glutaraldehyde,[80] by polymerization of either acrylamide[81] or vinyl alcohol[82, 83] in the presence of the enzyme, by impregnation of pre-swollen collagen with the enzyme,[84] and by ionic adsorption on to diethylamino-acetylcellulose.[85]

Fucosidases

One of the four forms of β-galactosidase separable from an extract of porcine kidney by gel filtration exhibited β-D-fucosidase activity.[86] Both the β-D-fucosidase and β-galactosidase activities of this fraction exhibited the same broad pH optimum (4.0—7.5), and pH and thermal stabilities,

[75] K. Lendzian and E. Schafer, *Phytochemistry*, 1973, **12**, 1227.
[76] M. Pascal and R. Dedonder, *Carbohydrate Res.*, 1972, **24**, 365.
[77] J. C. Tkacz and J. O. Lampen, *J. Bacteriol.*, 1973, **113**, 1073.
[78] V.V. Iurkevich and N. S. Kovaleva, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 1233.
[79] T. Kobayashi and M. Moo-Young, *Biotechnol. and Bioeng.*, 1973, **15**, 47.
[80] K. Venkatasubramanian and W. R. Vieth, *Biotechnol. and Bioeng.*, 1973, **15**, 583.
[81] H. Maeda, A. Yamauchi, and H. Suzuki, *Biochim. Biophys. Acta*, 1973, **315**, 18.
[82] H. Maeda, H. Suzuki, and A. Yamauchi, *Biotechnol. and Bioeng.*, 1973, **15**, 607.
[83] H. Maeda, H. Suzuki, and A. Yamauchi, *Biotechnol. and Bioeng.*, 1973, **15**, 827.
[84] S. S. Wang and W. R. Vieth, *Biotechnol. and Bioeng.*, 1973, **15**, 93.
[85] H. Maeda, H. Suzuki, and A. Sakimae, *Biotechnol. and Bioeng.*, 1973, **15**, 403.
[86] G. Y. Wiederschain and A. A. Prokopenkov, *Arch. Biochem. Biophys.*, 1973, **158**, 539.

and were inhibited to the same extents by D-galactose, D-fucose, and D-galactono-1,4-lactone. From the data obtained, it was concluded that one enzyme species catalyses the hydrolysis of 4-nitrophenyl β-D-galactopyranoside and 4-nitrophenyl β-D-fucopyranoside.

The existence of two forms (molecular weights 1.35×10^5 and 2×10^5) of α-L-fucosidase in porcine kidney has been demonstrated.[87] The two enzymes also differed in their thermal stability, dependence of activity on pH, pH stability during storage, and other characteristics. Both enzymes were able to cleave 4-nitrophenyl α-L-fucopyranoside, *O*-α-L-fucopyranosyl-(1 → 2)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose, *O*-α-L-fucopyranosyl-(1 → 2)-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-[α-L-fucopyranosyl-(1 → 3)]-D-glucose, and *O*-β-D-galactopyranosyl-(1 → 3)-*O*-[α-L-fucopyranosyl-(1 → 4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose. However, only the enzyme of lower molecular weight was also capable of hydrolysing *O*-β-D-galactopyranosyl-(1 → 4)-*O*-[α-L-fucopyranosyl-(1 → 3)]-D-glucose and *O*-β-D-galactopyranosyl-(1 → 4)-*O*-[α-L-fucopyranosyl-(1 → 3)]-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose. The action of the enzymes on other substrates was also investigated, and the specificity of the α-L-fucosidases was discussed with respect to the types of linkage cleaved and the nature of the carbohydrate units to which the L-fucose residues were bound.

The α-L-fucosidase activity of normal and scrapie-affected mouse brain has been investigated, and a pH optimum of 4.1 was found for the enzyme in both instances.[48] The activity of the enzyme in the affected brain was shown to be significantly greater than normal.

One (pH optimum 5.5) of the two β-galactosidases found in a liver extract of the marine gastropod *Turbo cornutus* was purified by way of fractional precipitation, heat and pH-variation treatments, ion-exchange chromatography, and gel filtration. It was shown to possess β-D-fucosidase, β-galactosidase, β-glucosidase, and α-L-arabinofuranosidase activities, in the ratio 13 : 5 : 5 : 1, using the corresponding 4-nitrophenyl glycosides as substrates.[69] The enzyme is regarded as a glycosidase capable of hydrolysing glycosidic bonds involving a terminal, non-reducing unit with the same configuration at C-1, C-2, and C-3 as β-D-galactopyranose.

The α-D-fucosidase present in dormant seeds of *Cajanus indicus* has been purified from an extract by gel filtration; this procedure yielded the enzyme in a fraction separate from the α-galactosidase and β-L-arabinosidase activities.[71]

α-D-Fucosidase activity has been detected in Takadiastase (an enzyme solution produced commercially from *Aspergillus oryzae*) using 4-nitrophenyl α-D-fucopyranoside as substrate.[88] Purification of the enzyme by fractionation with acetone, ion-exchange chromatography, and gel

[87] G. Y. Wiederschain and A. A. Prokopenkov, *Biokhimiya*, 1973, **38**, 113.
[88] S. Iwashita and F. Egami, *J. Biochem.* (*Japan*), 1973, **73**, 1217.

filtration gave a preparation free from all other glycosidases, with the exception of α-D-galactosidase. Further experiments showed that the hydrolyses of 4-nitrophenyl α-D-fucopyranoside and 4-nitrophenyl α-D-galactopyranoside are catalysed by a single enzyme (pH optimum 4.5—5.5); the K_m values for hydrolyses of the two substrates were 3.7 and 7.7 mmol l^{-1}, respectively. The D-fucoside was hydrolysed much more rapidly than the D-galactoside by the enzyme under identical conditions. The α-D-fucosidase activity was inhibited by 4-chloromercuribenzoate and silver nitrate, but not by H_4edta, copper(II) sulphate, or zinc sulphate. The enzyme was also found to be competitively inhibited by D-fucose and D-galactose.

Galactosidases

The detection, measurement, isolation, physical properties, chemical analysis, specificity of action, kinetic properties, mechanism of action, and physiological significance of the α-galactosidases have been reviewed.[89]

A general method for continuously monitoring the spectrophotometric assay of galactosidases at all values of pH using 4-nitrophenyl D-galactopyranosides as substrates has been reported.[17] The method was demonstrated specifically by the development of routine assays for the α-galactosidases from figs and *Mortierella vinacea* using 4-nitrophenyl α-D-galactopyranoside at pH 3.9 and 5.8, respectively. The wavelength of light at which the measurements are made can be selected according to the information required, *e.g.* maximum sensitivity at a selected pH, determination of the pH optimum of the enzyme with a pH-independent, difference-extinction coefficient, or the reduction of background absorbance for kinetic studies at high concentrations of substrate. Application of a computer to automating a recording polarimeter for β-galactosidase assays has been reported (see p. 358).[18]

1-Aminoglycosides have been reported to represent a new class of specific and relatively potent inhibitors of glycosidases.[23] The compounds are specific in their action for enzymes that act upon the glycoside corresponding to the glycone of the inhibitor molecule. For example, α- and β-galactosidases were inhibited by α-D-galactopyranosylamine, but by neither β-D-glucopyranosylamine nor β-D-mannopyranosylamine. The 'galactocerebrosidase' and β-galactosidase activities of tissues from a variety of clinical conditions in infants have been determined.[33]

The β-galactosidase responsible for degradation of the monogalactosyldiglyceride present in human brain has been shown to be severely deficient in the brain, liver, and skin fibroblasts obtained from cases of Krabbe's disease.[90] The β-galactosidase active towards galactocerebroside and psychosine was also found to be severely deficient. These findings have

[89] P. M. Dey and J. B. Pridham, *Adv. Enzymol.*, 1972, **36**, 91.
[90] D. A. Wenger, M. Sattler, and S. P. Markey, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 680.

provided new information on the substrate-recognition pattern of the enzyme and on the aetiology of globoid-cell leukodystrophy. The behaviour of β-galactosidase as a lysosomal hydrolase in eye tissues has been investigated.[91]

The β-galactosidase activity of human-skin fibroblasts from normal individuals has been investigated.[92] Two K_m values for the activity could be derived from Lineweaver–Burk plots, suggesting the presence of two enzyme species. A study of the effects of sodium chloride, heat, and 4-chlorophenylmercurisulphonate on the activity of the enzyme supported the view for the existence of two distinct populations of enzymes. A new method for extraction and assay of β-galactosidase (as a lysosomal glycosidase) in human skin has been reported.[34] Biopsies from normal skin were frozen, sectioned on a cryostat, and homogenized; the enzyme was demonstrated to be stable under the conditions used for extraction, and its kinetic characteristics were evaluated. The specific activity of the isolated β-galactosidase was found to be as much as ten-fold higher than that previously reported. The levels of β-galactosidase activity in homogenates of fibroblasts derived from normal individuals, from patients affected with disorders attributable to a deficiency of α-L-iduronidase (Hurler syndrome, Scheie syndrome, and a Hurler–Scheie compound condition), and from the parents of these patients have been determined and compared with the levels of other glycosidases in the homogenates.[38] The β-galactosidase activities of the fibroblasts were found to provide a useful basis for normalization of the α-L-iduronidase activities, since the overlap of normal and heterozygous levels of α-L-iduronidase was thereby reduced sufficiently to be of diagnostic value.

The action pattern of a partially purified galactosylgalactosylglucosylceramidase α-galactosidase from human liver has been studied using a galactosylgalactosylglucosylceramide tritiated in the terminal, non-reducing D-galactose unit as substrate.[93] Hydrolysis of the substrate was absolutely dependent on a mixture of sodium taurocholate and Triton X-100, and was markedly inhibited by either human serum albumin or sodium chloride. *O*-β-D-Galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide inhibited the hydrolysis at all concentrations of substrate, whereas digalactosylceramide stimulated the hydrolysis at low concentrations of substrate, but inhibited the hydrolysis at high concentrations of substrate. α-Galactosidase activity, as measured against 4-methylumbelliferyl α-D-galactopyranoside, followed the activity against the natural substrate throughout fractionation procedures, and the two activities exhibited identical characteristics towards inactivation by heat. Characteristics for hydrolysis of the synthetic substrate differing from those for the natural substrate included the pH optimum, the shape of the Lineweaver–Burk plot, and the effects of inhibitors and activators. Mutual inhibition of hydrolysis of the

[91] B. S. Kasavina and N. B. Chesnokova, *Doklady Akad. Nauk S.S.S.R.*, 1973, **209**, 984.
[92] J. N. Kanfer and C. Spielvogel, *Biochim. Biophys. Acta*, 1972, **289**, 359.
[93] M. W. Ho, *Biochem. J.*, 1973, **133**, 1.

two substrates was predominantly non-competitive. The results of these investigations were discussed in the light of special problems associated with the hydrolysis of glycolipids in aqueous media.

The antagonistic actions of chondroitin sulphate and cetylpyridinium chloride on human-liver β-galactosidase have been investigated.[94] Cetylpyridinium chloride completely inhibited the action of the enzyme, whereas shark-cartilage chondroitin sulphate only partially inhibited the activity. However, when cetylpyridinium chloride was added to a mixture of liver extract and chondroitin sulphate, there was complete restoration of activity. In contrast, addition of chondroitin sulphate to a mixture of the enzyme and cetylpyridinium chloride failed to restore the activity. It was presumed that inhibition of enzymic activity by chondroitin sulphate is due to the formation of a stable complex, which could be dissociated by cetylpyridinium chloride. On the other hand, since cetylpyridinium chloride is a strongly irreversible denaturing agent for the enzyme alone, this process could not be reversed by subsequent addition of the polysaccharide. The structure of a keratan-sulphate-like material in the liver of a patient with G_{M1}-gangliosidosis (β-galactosidase deficiency) has been investigated.[95]

The β-galactosidase and several other glycosidase activities of the small intestines of humans and of non-human primates (Lorisidae, Hapalidae, and Cebidae) have been determined using lactose as substrate.[73] The awe monkey (*Aotus trivirigatus*) is found to have the highest β-galactosidase activity. In humans with normal β-galactosidase activity, the concentration of β-galactosidase is greater than those of either an α-glucosidase active against palatinose or αα-trehalase, whereas in humans and non-human primates with an isolated β-galactosidase deficiency, the concentrations of the latter two enzymes are greater than that of β-galactosidase.

Thermolabile (*A*) and thermostable (*B*) α-galactosidases have been separated and purified from human placenta.[96] Enzyme *A* (molecular weight 1.5×10^5) was obtained in homogeneous form, whereas enzyme *B*, which possesses the same molecular weight as *A*, was not obtained in a completely pure form. Both enzymes exhibited maximum activity at pH 4.5, *A* possessing a broad peak and *B* a sharp peak in the pH–activity curves. Enzyme *A* (p*I* 4.7) was active against 4-methylumbelliferyl α-D-galactopyranoside and melibiose (K_m values 3.4 and 40.6 mmol l^{-1}, respectively), whereas enzyme *B* (p*I* 4.4) exhibited first-order kinetics in hydrolysing the synthetic substrate, but was inactive against melibiose. Enzyme *A* could be inhibited by *myo*-inositol, but enzyme *B* could not. Antibodies against the two enzymes were produced, but did not cross-react. Neither antiserum neutralized enzymic activity. Treatment of

[94] J. A. Kint, *F.E.B.S. Letters*, 1973, **36**, 53.
[95] G. C. Tsay and G. Dawson, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 759.
[96] E. Beutler and W. Kuhl, *J. Biol. Chem.*, 1972, **247**, 7195.

enzyme *A* with neuraminidase did not alter either its immunological or kinetic characteristics, and, thus, the studies do not support the concept that α-galactosidase *A* is the neuraminosyl derivative of *B*, or that the two enzymes are closely related.

4-Aminophenyl melibioside reacts through its amino-group with a derivatized agarose, and the product has been shown to be a selective adsorbent for galactosylgalactosylglucosylceramidase and other α-galactosidases, and was employed in isolating these enzymes from partially pure fractions of human plasma.[97] Quantitative recovery of the enzyme from the affinity-chromatography column was readily achieved by the addition of detergent to the eluting buffer. The behaviour of multiple forms of human galactosylgalactosylglucosylceramidase on affinity chromatography has been investigated for kidney, plasma, and urine samples without prior purification. A total of five different forms of the enzyme were observed in normal plasma, together with six forms of α-galactosidase that are specific for 4-nitrophenyl α-D-galactopyranoside. The same pattern of α-galactosidases was obtained for urine concentrates, whereas cholate-solubilized lysosomal enzymes from kidney included only one form of galactosylgalactosylglucosylceramidase. Three of the plasma galactosylgalactosylglucosylceramidases exhibited optimal activity at pH 7.2, but these forms were shown to be absent in the plasma of heterozygous and hemizygous patients with Fabry's disease. The amounts of the other two forms of plasma galactosylgalactosylglucosylceramidase (pH optima 5.4) were diminished in heterozygotes and particularly in hemizygotes. Only one of the six α-galactosidases active against the synthetic substrate is deficient in the plasma from a case of Fabry's disease. Several additional proteins with affinity for the ligand were obtained from the plasma of Fabry patients, and it was suggested that these species are inactive forms of galactosylgalactosylglucosylceramidase. The same type of affinity-chromatography column has been used to purify an α-galactosidase that hydrolyses both galactosylgalactosylceramide and 4-methylumbelliferyl α-D-galactopyranoside.[98] The preparation was partially separated into two enzymically active proteins by either isoelectric focusing or electrophoresis on cellulose acetate. Of two proteins similarly obtained from the plasma of a case of Fabry's disease, only the protein of lower electrophoretic mobility possessed detectable enzymic activity. The results indicated that accumulation of galactosylgalactosylceramide in certain organs of cases of Fabry's disease is due to the alteration of an enzyme.

Four forms of β-galactosidase active against 4-nitrophenyl β-D-galactopyranoside have been separated from an extract of porcine kidney by gel filtration.[86] Three of the enzymes were also active against lactose, whereas the fourth was inactive against lactose but active against a β-D-fucopyranoside. The latter β-galactosidase and the β-D-fucosidase activities

[97] C. A. Mapes and C. C. Sweeley, *J. Biol. Chem.*, 1973, **248**, 2461.
[98] C. A. Mapes and C. C. Sweeley, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 1317.

possessed similar broad pH optima (4.0—7.5), pH stability, and thermostability, and were inhibited to similar extents by D-galactose, D-fucose, and D-galactono-1,4-lactone. It was concluded from these data that both the β-D-fucoside and β-D-galactoside substrates are cleaved by the same enzyme. Another report on the galactosidases of porcine kidney has described the occurrence of four β-galactosidases and two α-galactosidases.[87]

The β-galactosidase activities of the small intestines of the cat, guinea pig, rabbit, mouse, and rat have been studied.[99] High activity was recognized in the jejunal segments of all the species studied, and also in the small-intestinal mucosa of guinea pigs. The effects of feeding and starvation upon the level of the enzyme were investigated.

Intraperitoneal administration of deoxycorticosterone to rabbits caused alterations in the levels of activity of several carbohydrases in the vitreous body of the eye, including a fall in the level of β-galactosidase.[100] These enzymes are suggested to participate in the mechanism whereby the intraocular pressure is increased by deoxycorticosterone.

A hetero-β-galactosidase activity has been identified in the small-intestinal mucosa of rabbits.[101] The enzyme, which is localized in the cytosol fraction, has a pH optimum at neutrality and a low molecular weight. The enzyme was active against synthetic β-D-galactopyranosides and β-D-glucopyranosides, but was only slightly active against lactose. Inhibition of the enzyme was effected with 4-chloromercuribenzoate, but not by lactose. The distribution of the enzyme was investigated, and administration of colchicine was paralleled by depressions of the levels of hetero-β-galactosidase and neutral lactase β-galactosidase. The developmental patterns of the two activities diverge significantly, and immunological studies did not reveal any cross-reaction between them. No precursor–product relationship between the two enzymes could be established.

The effects of metal ions on the acidic and neutral glycosidases (including the β-galactosidases) of a rabbit-liver extract have been investigated.[102] Two galactosidases present in the extract were inhibited by Ag^+ and Hg^+ ions, but, whereas Cu^{2+} ions activated the acidic β-galactosidase, they strongly inhibited the neutral enzyme. Zn^{2+} and Cd^{2+} ions slightly activated the acidic β-galactosidase, but had no effect on the neutral enzyme. Neither β-galactosidase was affected by H_4edta.

The changing concentrations of β-galactosidase and β-glucuronidase in the brain, heart, and liver of mice have been found to be co-ordinated during the development of the organs.[103] Although co-ordinated, the individual development patterns of the two enzymes were found to be

[99] E. Hietanen, *Comp. Biochem. Physiol.*, 1973, **46A**, 359.
[100] B. S. Kasavina, P. V. Sergeev, N. B. Chesnokova, and L. M. Konstantinova, *Biull. Eksp. Biol. i Medit.*, 1973, **76**, 48.
[101] J. D. Johnson, *Biochim. Biophys. Acta*, 1973, **302**, 382.
[102] I. Suzuki and H. Kushida, *J. Biochem.* (*Japan*), 1973, **74**, 627.
[103] M. Meisler and K. Paigæn, *Science*, 1972, **177**, 894.

under independent control by genetic elements, apparently linked to the respective structural genes. The α- and β-galactosidases of both normal mouse brain and scrapie-affected mouse brain have been shown to exhibit optimum activity at pH 4.1 and 3.5, respectively.[48]

A preparation of β-galactosylceramidase from rat liver has been tested for its susceptibility to potential inhibitors that structurally resemble substrates for the enzyme.[104] The amide made from 2-hydroxydecanoic acid and DL-*erythro*-3-phenyl-2-aminopropane-1,3-diol proved to be a good inhibitor of the enzyme, and acted non-competitively. Replacement of any of the three hydroxy-groups with hydrogen atoms reduced the effectiveness of the inhibitor, as did inversion of the configuration at C-3 or substitution of the aromatic ring. *N*-Acetyl- and *N*-(n-hexyl)-psychosines were effective inhibitors of the mixed type, whereas the longer-chain homologue *N*-decanoylpsychosine was both a competitive inhibitor and a substrate for the β-galactosylceramidase. Lactosylceramide, a naturally occurring glycolipid, was a competitive inhibitor of modest efficiency, whereas D-galactono-1,4-lactone proved to be an excellent competitive inhibitor. From the results of these comparative studies, it was concluded that the active site of the enzyme binds only substances containing or resembling D-galactose, whereas the secondary effector site binds a variety of substances possessing the central nitrogeneous core-region of cerebrosides.

Matrices for the purification of rat-liver glycosidases by affinity chromatography have been produced by the reaction of a number of amines and 4-aminophenyl β-D-glycosides with pre-derivatized Sepharose.[52] Of the potential affinants investigated, one derived from 4-aminophenyl β-D-galactopyranoside was found to be suitable for the purification of β-galactosidase; the adsorbed enzyme was subsequently desorbed by elution with sodium chloride solution. Analogous affinants prepared from 4-aminophenyl β-D-glucopyranuronoside and 4-aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside did not adsorb the enzyme.

Injection of rats with cystamine produced a wave-like increase in the β-galactosidase activity of the plasma.[105] Each wave of activity was preceded by changes of the levels of enzyme activity in the liver and spleen. A plasma-membrane fraction isolated from the 4-dimethylaminoazobenzene-induced rat hepatoma D23 has been investigated by immunodiffusion methods combined with staining reactions for β-galactosidase activity.[53] The presence of the enzyme was established, and the enzyme-active antigen was also found, with varying distribution, in the tumour microsomes.

The β-galactosidase activity of different parts of the male reproductive tract of the giant octopus (*Octopus dofleini martini*) has been determined; the spermatophore and the 'prostate' were shown to be major sources of the enzyme.[54]

[104] R. C. Arora, Y. N. Lin, and N. S. Radin, *Arch. Biochem. Biophys.*, 1973, **156**, 77.
[105] R. Gol-Winkler and R. Goutier, *Biochem. Pharmacol.*, 1973, **22**, 1431.

Two β-galactosidase activities (pH optima 3.0 and 5.5) have been detected in a liver extract of the marine gastropod *Turbo cornutus*.[69] The enzymes were purified by means of fractional precipitation, heat and pH-variation treatments, ion-exchange chromatography, and gel filtration. The purified enzyme (pH optimum 5.5) possessed β-galactosidase, β-glucosidase, β-D-fucosidase, and α-L-arabinofuranosidase activities, in the ratio 5 : 5: 13 : 1, when 4-nitrophenyl glycosides were used as substrates. The enzyme is regarded as a glycohydrolase that hydrolyses glycosidic bonds involving a terminal, non-reducing residue with the same configuration at C-1, C-2, and C-3 as β-D-galactopyranose. The other enzyme (pH optimum 3.0) was inhibited by cyanide ion, but was resistant to the inhibitory action of such compounds as copper(II) sulphate and mercury(II) chloride, whereas the former enzyme was inhibited both by cyanide and heavy-metal ions. Both enzymes were resistant to the action of sodium azide and H_4edta. Other properties (including the stabilities to heat and pH) and the K_m values for the enzymes were also assessed.

The inhibition of the β-galactosidase, β-glucosidase, β-xylosidase, and α-L-arabinosidase activities of sweet almond emulsin by synthetic *C*-glycosides has been investigated.[70] The inhibition was shown to be non-competitive in each case, and the effects of the inhibitors decreased in the order 1-β-D-glucopyranosyl-, 1-α-L-arabinosyl-, 1-β-D-galactopyranosyl-, and 1-β-D-xylopyranosyl-4-nitrobenzene. The observations were interpreted as evidence that the four enzyme activities are manifested by a single enzyme species.

The α-galactosidase of dormant seeds of *Cajanus indicus* has been purified and separated from α-D-fucosidase and β-L-arabinosidase present in the extract by gel filtration.[71] Purification of α- and β-galactosidases from germinating seeds of *Dolichos biflorus*, by means of ion-exchange chromatography, has been described.[57] The enzymes exhibited optimal activity at pH 5.0 and 3.9, respectively, and their storage properties were investigated.

The α-galactosidase activities in extracts from the endosperm and embryo of fenugreek seeds (*Trigonella foenum-graecum*) have been determined at various stages of germination.[106] The onset of the breakdown of galactomannan coincided with the appearance of α-galactosidase and β-mannosidase activities; these activities increased throughout the period of degradation of the galactomannan and then remained constant.

The α-galactosidase of ficin and the β-galactosidase of Jack-bean meal have been purified by affinity chromatography on a D-galactonic acid derivative of Sepharose.[107] In view of the success achieved, it was suggested that the use of sugar lactones coupled to an inert support might be generally applicable to the purification of lysosomal 'acid' glycohydrolases.

[106] J. S. G. Reid and H. Meier, *Planta*, 1973, **112**, 301.
[107] J. N. Kanfer, G. Petrovich, and R. A. Mumford, *Analyt. Biochem.*, 1973, **55**, 301.

Considerable amounts of β-galactosidase activity were induced when the mycelia of *Aspergillus nidulans* were grown with D-galactose or lactose as the carbon source.[108] The effects of various conditions of growth on production of the activity were investigated. Gel filtration and sedimentation experiments with a density gradient of sucrose revealed the presence of two protein forms of the enzyme. Molecular weights of 1.2×10^5 and 4.5×10^5 were derived from the latter experiments, and it appeared that the two forms are different aggregates of the same polypeptide possessing a monomer–tetramer relationship. The β-galactosidase of *A. niger* has been purified by affinity chromatography on porous glass to which the inhibitor 4-aminophenyl 1-thio-β-D-galactopyranoside had been attached.[109] The β-galactosidase was competitively inhibited by D-galactose, but not by D-glucose. The enzyme was immobilized by coupling to glass *via* a diazo-linkage; the pH optimum, temperature optimum, and stability of the enzyme were not affected by insolubilization.

Purification of the α-D-fucosidase from the commercial Takadiastase obtained from *A. oryzae*, by means of precipitation with acetone, ion-exchange chromatography, and gel filtration, freed the enzyme from all glycosidase activities, with the exception of α-D-galactosidase.[88] Further experiments showed that the hydrolyses of α-D-fucosides and α-D-galactosides by the purified enzyme are catalysed by a single species, which possessed K_m values of 3.7 and 7.7 mmol l^{-1} for the two 4-nitrophenyl glycopyranosides, respectively; 4-nitrophenyl α-D-fucopyranoside was hydrolysed more rapidly than the corresponding α-D-galactopyranoside. The enzymic activity was inhibited by silver nitrate and 4-chloromercuribenzoate, but not by H_4edta, copper(II) sulphate, or zinc sulphate. The crude Takadiastase was also found to contain another α-galactosidase that possesses no α-D-fucosidase activity.

The β-galactosidase from *Bacillus megaterium* has been purified by affinity chromatography on a column of Sepharose, which had been derivatized and then allowed to react with the amino-group of 4-aminophenyl 1-thio-β-D-galactopyranoside.[110] The enzyme was found to have a dimeric subunit structure, with the monomer having a molecular weight of 1.2×10^5. The equilibrium constant of monomer–dimer interconversion favoured dissociation in the isolated state. This dissociation was minimized by incorporation of 5% sucrose into the buffer maintained at a temperature of 5 °C. Molecularly homogeneous monomer ($S_{20,w} = 3$) and dimer ($S_{20,w} = 8$) could be obtained by sucrose density-gradient centrifugation. The activity of the monomer was studied both by urea-dissociation experiments and by immobilization of the monomer on Sepharose by covalent attachment. Although the immobilized monomer was inactive, it was still capable of binding the inhibitor 4-aminophenyl

[108] P. A. Fantes and C. F. Roberts, *J. Gen. Microbiol.*, 1973, **77**, 471.
[109] J. H. Woychik and M. V. Wondolowski, *Biochim. Biophys. Acta*, 1972, **289**, 347.
[110] H. B. Pollard and E. Steers, *Arch. Biochem. Biophys.*, 1973, **158**, 650.

1-thio-β-D-galactopyranoside, and could be activated by the addition of free monomer. Free monomers from the β-galactosidase of *Escherichia coli* were able to form active hybrids with the immobilized *B. megaterium* monomer; monomers of the two enzymes were found to possess similar amino-acid compositions. From these studies, it was concluded that the free monomeric form of *B. megaterium* is inactive and that the dimer is the active species, in contrast to the β-galactosidase from *E. coli*, where only the tetrameric species is active.

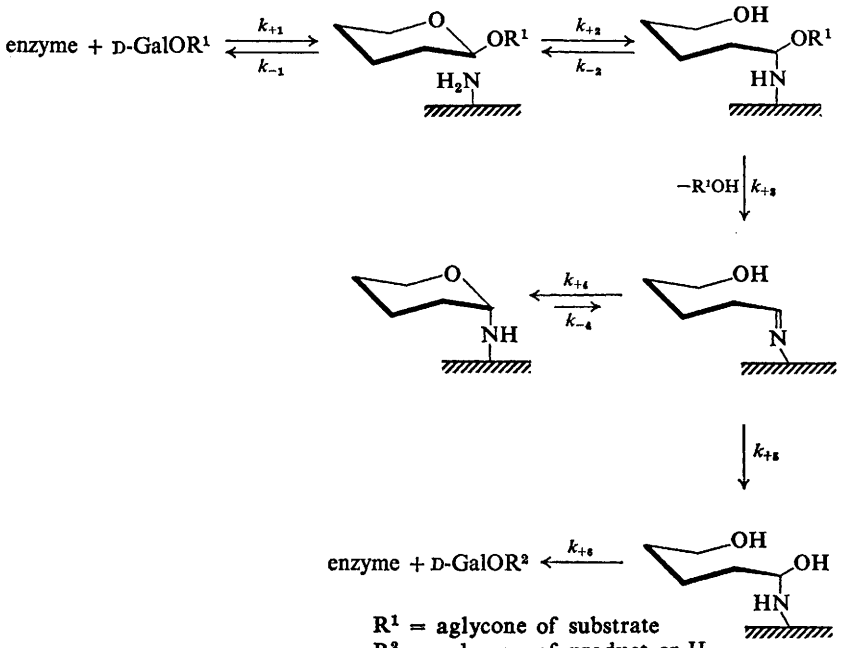
An α-galactosidase from culture filtrates of *Corticium rolfsii* has been obtained in a form free from other glycosidases.[111] The activity of the enzyme towards a number of synthetic and oligo-D-galactosyl substrates was investigated. The enzyme exhibited maximal activity in the pH range 2.5—4.5, and was completely inhibited by Hg^{2+} and Ag^{+} ions.

An acid β-galactosidase has been purified from culture filtrates of *C. rolfsii* by a combination of ion-exchange chromatography on QAE- and SP-Sephadex.[112] Maximal activity of the enzyme towards 4-nitrophenyl β-D-galactopyranoside occurred at pH 2.0—2.5, and the enzyme was appreciably active at pH 1.5—1.8. In contrast to its high activity towards the synthetic glycoside, the enzyme exhibited little reactivity towards lactose. The enzyme was stable in the pH range 2—8 and was inhibited by Hg^{2+} ions.

It has been reported that the spin-labelled compound 2,2,6,6-tetramethyl-4-[^{3}H]piperidin-4-olyl-1-oxyl β-D-galactopyranoside is a substrate for β-galactosidase and that it is accumulated by strains of *Escherichia coli*.[113] Induction of a strain of the organism with isopropyl 1-thio-β-D-galactopyranoside resulted in a hundred-fold increase in the uptake of the spin-labelled D-galactoside. Binding of the spin-labelled compound to membranes derived from the organism was inhibited by lactose. Purification of the β-galactosidase from *E. coli* by affinity chromatography on an agarose derivative, which had reacted with 4-aminophenyl 1-thio-β-D-galactopyranoside, has been investigated for both normal adsorption–desorption (two-step) conditions and elution conditions giving selective retardation (single-step).[114] Products of 60 and 77% purity, respectively, were obtained from the two procedures. The effects of flow rate and ionic strength of the eluent on the binding of the enzyme by the affinity material were investigated for adsorption and desorption processes. The operational stability of the adsorbent was examined under conditions of continuous use and re-use, and it was found that the binding capacity was unaltered after thirty-five uses of the column. The single-step elution technique was discussed in relation to large-scale preparations of the enzyme and to its use in continuous affinity chromatography.

[111] A. Kaji and O. Yoshihara, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1335.
[112] A. Kaji, M. Sato, N. Shinmyo, and M. Yasuda, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1729.
[113] W. G. Struve and H. M. McConnell, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1631.
[114] P. J. Robinson, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1972, **285**, 28.

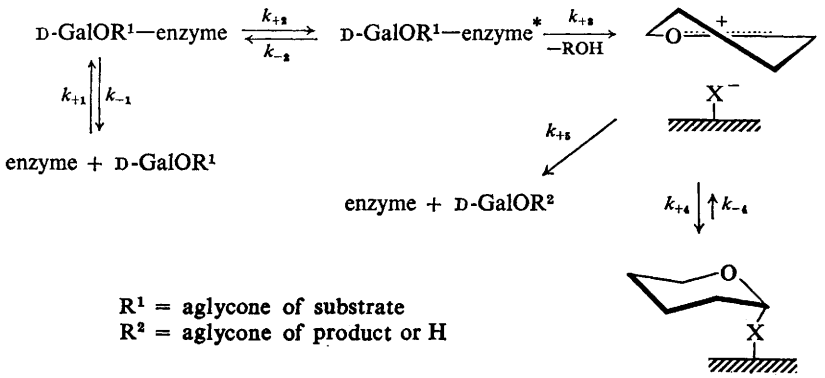
Steady-state kinetic parameters for the hydrolysis of thirteen aryl β-D-galactopyranosides catalysed by a β-galactosidase from *E. coli* showed no simple dependence on the acidity of the aglycone.[115] Kinetic isotope effects (k_H/k_D) for seven of the substrates varied from 1.0 for poor substrates to 1.25 for hydrolysis of the D-galactosyl-enzyme. Methanolysis increased k_H/k_D for the removal of D-galactosyl residues by the enzyme, but had no effect in the cases of poor substrates. These data are considered to be incompatible with a two-step mechanism for the reaction catalysed by the enzyme. A complex, ring-opening mechanism (Scheme 3) is



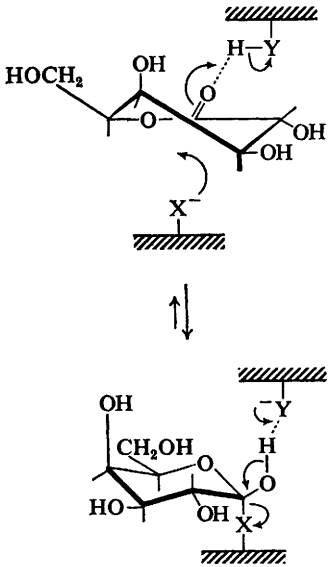
Scheme 3

considered to be required if neither a change of conformation nor non-productive binding nor a breakdown of the principle of microscopic reversibility is assumed. A preferred pathway (Scheme 4), involving a conformational change and liberation of a D-galactopyranosyl cation as an intimate ion-pair *etc.*, was also proposed. The latter scheme was used to rationalize previously uninterpretable data for the enzymic mechanism. A process analogous to ion-pair collapse has been invoked to explain the greater affinity of β-galactosidases for D-galactono-1,5-lactone than for 1-thio-β-D-galactopyranosides (Scheme 5).

[115] M. L. Sinnott and I. J. L. Souchard, *Biochem. J.*, 1973, **133**, 89.



Scheme 4



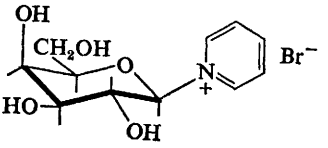
Scheme 5

Although the acid-catalysed hydrolyses of t-butyl, 1,1-dimethylpropyl, and diphenylmethyl β-D-galactopyranosides are claimed to proceed in part by alkyl–oxygen bond fission, products derived from such a process were not detected in hydrolyses of these substances by the β-galactosidase of wild-type *E. coli*.[116] The effect of methanol on the enzyme-catalysed hydrolysis of a number of derivatized nitrophenyl β-D-galactopyranosides by the β-galactosidase from *E. coli* has been studied under steady-state

[116] M. Maybury and M. L. Sinnott, *J.C.S. Perkin II*, 1973, 300.

conditions.[117] The initial fractional rate of increase of the rate constant (k_{cat}) as a function of the concentration of methanol indicated that degalactosylation of the enzyme is rate-determining when 2,4- and 3,5-dinitrophenyl β-D-galactopyranosides are used as substrates. The decrease in k_{cat} at high concentrations of methanol for these substrates is considered to arise from causes other than D-galactosylation of the enzyme becoming rate-limiting. Both galactosylation and degalactosylation of the enzyme required protonation of a group of pK_a 9 within the enzyme molecule.

β-D-Galactopyranosyltrimethylammonium bromide has been shown to be a competitive inhibitor of the β-galactosidase of *E. coli*, whereas tetramethylammonium bromide caused no inhibition.[118] The kinetics of deactivation of the Mg^{2+}-saturated enzyme in H_4edta, both in the presence and in the absence of β-D-galactopyranosyl tetramethylammonium bromide, were found to be similar. The results led to the conclusions that Mg^{2+} ions and the inhibitor bind independently, and that Mg^{2+} ions do not act as an electrophilic catalyst. Complexant fluorescence measurements indicated that one Mg^{2+} ion is bound per molecular weight of 1.35×10^5, and this stoicheiometry was confirmed by equilibrium dialysis. 1,6-Anhydro-β-D-galactopyranose functioned as neither a substrate nor an inhibitor of the *E. coli* β-galactosidase. Consideration of the conformations available to the cationic inhibitor and the anhydro-sugar indicated that the former is bound to the enzyme with the pyranose ring in a conformation not greatly different from the normal $^4C_1$ conformation. Whereas most theories concerning the mechanism of action of glycosidases invoke some form of general acid or electrophilic catalysis for departure of the aglycone, it has been reported that the β-D-galactopyranosylpyridinium cation (1)



(1)

is nonetheless hydrolysed at the active site of the β-galactosidase of *E. coli*.[119] Kinetic data confirming that the hydrolysis is effected at the conventional active site of the enzyme were presented. It was implied that conformational distortion and either electrostatic or nucleophilic catalysis must be responsible for the entire catalytic effect for hydrolysis of the pyridinium salt by the enzyme. It was argued that some form of partial proton-transfer to the π-electrons of the pyridine ring, as well as being intrinsically unlikely, would not assist in cleavage of the glycosyl–nitrogen bond, which is orthogonal to this system.

[117] M. L. Sinnott and O. M. Viratelle, *Biochem. J.*, 1973, **133**, 81.
[118] G. S. Case, M. L. Sinnott, and J.-P. Tenu, *Biochem. J.*, 1973, **133**, 99.
[119] M. L. Sinnott, *J.C.S. Chem. Comm.*, 1973, 535.

The β-galactosidase activity found in mycelia of *Neurospora crassa* grown on cellobiose has been investigated.[120] The enzyme, which exhibited maximal activity at pH 6, was found to be more sensitive to inhibition by cellobiose than by lactose. Precipitation of the enzyme by ammonium sulphate was investigated. The characteristics of the enzyme distinguished it from the previously identified β-galactosidases (pH optima 4 and 7) of *N. crassa*, indicating that a third β-galactosidase is present in mycelia grown on cellobiose.

Immobilized forms of β-galactosidase have been prepared by covalent attachment to activated forms of iron oxide and cellulose-entrapped iron oxide,[121] by encapsulation during the polymerization of acrylamide [81, 122] and the copolymerization of 1,6-diaminohexane and terephthaloyl dichloride,[123] and by adsorption on to phenolic resins.[124]

β-Glucosidases

1-Aminoglycosides have been reported to represent a new class of specific and relatively potent inhibitors of glycosidases; the compounds are specific inhibitors of enzymes that act upon glycosides corresponding to the glycone of the inhibitor.[23] Thus the α-glucosidase of *Saccharomyces oviformis* and the β-glucosidases of almond emulsin and *Kluyveromyces fragilis* were inhibited by β-D-glucopyranosylamine, but not by the stereoisomeric α-D-galactopyranosyl- or β-D-mannosyl-amines. The exceptional stability of the glycosidic linkage in 1-glycopyranosyl-imidazoles, which also act as glycosidase inhibitors, renders them of particular value for studies of glycosidase–inhibitor interactions.[24] In the case of glucosidases, inhibition by 1-α- and -β-D-glucopyranosyl-imidazoles of the hydrolysis of methyl α-D-glucopyranoside by yeast α-glucosidase exhibited a high degree of anomer specificity, whereas a lack of specificity for the anomers was apparent in the hydrolysis of cellobiose by almond β-glucosidase.

The hydrolysis of 2-dithiothreityl β-D-glucopyranoside by β-glucosidase has been investigated.[125]

A fluorometric procedure utilizing 4-methylumbelliferyl α-D-glucopyranoside as substrate has been described for the assay of α-glucosidase.[126] The method was shown to be particularly suitable for the assay of the enzyme in animal tissues and fluids. Using this method, the fibroblasts and liver from patients with Pompes disease were shown to be deficient in α-glucosidase activity, and the method is advocated for the study of

[120] C. B. Perry and G. Lester, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1476.
[121] P. J. Robinson, P. Dunnill, and M. D. Lilly, *Biotechnol. and Bioeng.*, 1973, **15**, 603.
[122] A. Dahlqvist, B. Mattiasson, and K. Mosbach, *Biotechnol. and Bioeng.*, 1973, **15**, 395.
[123] J. C. W. Østergaard and S. C. Martiny, *Biotechnol. and Bioeng.*, 1973, **15**, 561.
[124] W. L. Stanley and R. Palter, *Biotechnol. and Bioeng.*, 1973, **15**, 597.
[125] D. L. Storm, R. C. Buri, and W. Z. Hassid, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 147.
[126] I. S. Salafsky and H. L. Nadler, *J. Lab. Clin. Med.*, 1973, **81**, 450.

α-glucosidase activity in pre- and post-natal detection of the disease. A polarimetric assay for β-glucosidase has been used in conjunction with gel filtration and disc-gel electrophoresis of the enzyme, and the application of a computer to automation of the enzymic assay was described (see p. 358).[18] Spurious results have been found to arise from the determination of D-glucose in the presence of cellobiose *etc.* by glucose oxidase using a Glucostat special kit (Worthington Biochemical Corporation), owing to the presence of contaminating β-glucosidase.[127] This problem can be overcome by either a dilution technique or inactivation of the β-glucosidase by heat.

The β-glucosidase activities were investigated in a study of the lysosomal glycohydrolases of eye tissues.[91]

The glucocerebrosidase activities of tissues from cases of a variety of clinical conditions in infants have been investigated.[33] A deficiency in the activity of glucosylceramidase has been observed in skin fibroblasts and spleen tissues from cases of Gaucher's disease, compared with normal subjects.[128] Preliminary studies indicated the presence in the diseased spleen of a material similar to glucosylsphingosine, which was not detected in spleen tissues of normal and pathological controls. The subcellular distribution of 'acid' and 'neutral' α-glucosidases (pH optimum 4.0 and 6.5, respectively) has been investigated in biopsy specimens of human skeletal muscle obtained from normal subjects, from cases of α-glucosidase (maltase) deficiency in adults, and from a case of myophosphorylase deficiency.[129] The highest relative specific activities of 'acid' α-glucosidase and other 'acid' glycohydrolases were observed in light mitochondrial fractions.

The activities of α-glucosidase against maltose and palatinose (6-*O*-α-D-glucopyranosyl-D-fructose), of β-glucosidase against cellobiose, and of other glycosidases in the small intestines of humans and other non-human primates (Lorisidae, Hapalidae, and Cebidae) have been determined.[73] Black and red tamarins (*Tamarinus nigricollis*) were found to possess the highest concentrations of 'maltase'- and 'palatinase'-α-glucosidase. In humans with normal β-galactosidase activity, the concentrations of 'palatinase'-α-glucosidase and trehalase were found to be lower than that of β-galactosidase; however, in humans and non-human primates with an isolated deficiency of β-galactosidase, the 'palatinase'-α-glucosidase and trehalase activities were shown to be higher.

Concanavalin A inhibited the hydrolysis of dextrans and glycogen by the 'acid' α-glucosidase from porcine spleen, but had no effect on the hydrolysis of amylose and maltose by the enzyme.[130] The degree of inhibition depended on the ratio of concanavalin A and dextran used and on the structure of the dextran. The hydrolysis of linear dextrans was

[127] D. M. Pharr and D. B. Dickinson, *Analyt. Biochem.*, 1973, **51**, 315.
[128] S. S. Raghavan, R. A. Mumford, and J. N. Kanfer, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 256.
[129] C. Angelini and A. G. Engel, *Arch. Biochem. Biophys.*, 1973, **156**, 350.
[130] M. E. Preobrazhenskaya, *Biokhimiya*, 1973, **38**, 763.

inhibited up to 90%, whereas up to 60% inhibition was obtained with branched dextrans. Although these events occurred only at high concentrations of the enzyme, the different effects of the lectin led to the conclusion that the enzyme is probably not a glycoprotein.

The α-glucosidase activities of the small intestines of cats, rabbits, guinea pigs, mice, and rats have been investigated.[99] As with other glycosidases present, maximum activity was found in the jejunal segments of all the species. A comparatively low α-glucosidase activity was apparent in cats.

Intraperitoneal administration of deoxycorticosterone to rabbits caused alterations in the levels of activity of several carbohydrases in the vitreous body of the eye, including a fall in the level of β-glucosidase.[100] It is assumed that the enzyme participates in the mechanism whereby the intraocular pressure is increased following administration of deoxycorticosterone.

Rabbit muscles have been shown to contain different amounts of two α-glucosidases possessing pH optima at neutrality.[131] The fractions, which were separated by fractional precipitation with ammonium sulphate, were partially purified and their properties compared. Both exhibited identical substrate specificities and inhibition kinetics, and they could not be distinguished by these properties from a purified preparation of the major 'neutral' α-glucosidase. It was concluded that the two fractions represent the same activity associated with different cell components. The purified α-glucosidase, for which K_m values were determined for a number of oligosaccharides, exhibited optimal activity at pH 7.4, and was inhibited slightly by polyols, completely by 2-amino-2-hydroxymethylpropane-1,3-diol, and non-competitively by bivalent cations. By its action on maltodextrins and panose, the α-glucosidase was shown to possess constitutive 1,4- and 1,6-α-glucanhydrolase activities, although it had little action on glycogen. The enzyme is considered to be an oligosaccharidase, which acts on glycogen in conjunction with α-amylase. An ancillary role in controlling the structure of glycogen is proposed for the combined hydrolytic actions of the two enzymes.

A hetero-β-galactosidase, which has been identified in the small intestinal mucosa of rabbits, on partial purification and characterization, was active against synthetic β-D-glucopyranosides and β-D-galactopyranosides.[104] The enzyme was found to be localized in the cytosol fraction; it possessed a pH optimum at neutrality, a low molecular weight, and was not inhibited by either lactose or 4-chloromercuribenzoate.

The activity and intracellular distribution of several glycosidases in rabbit liver have been examined.[47] Glycosides of the steroid oestrogen that have been shown to occur naturally in rabbits were used as substrates, and the results were compared with those obtained using synthetic

[131] J. Carter and E. E. Smith, *Arch. Biochem. Biophys.*, 1973, **155**, 82.

4-nitrophenyl glycosides. The β-glucosidase activity was shown to be concentrated in a non-sedimentable fraction of the liver homogenate and it did not exhibit latency. No difference was found in the intracellular distribution of the enzyme activity towards the two types of substrate. Both the 'acidic' and 'neutral' α-glucosidases of rabbit liver were strongly inhibited by Hg^{2+} and Ag^{+} ions; Zn^{2+} ions slightly inhibited the latter, but not the former, enzyme, but the two enzymes generally showed no great differences in the effects elicited by added metal ions or H_4edta.[102]

Over the ranges examined, the α- and β-glucosidase activities of normal and scrapie-affected mouse brain displayed two peaks of enzyme activity (pH optima 4.1 and 6.5, and 4.1 and 5.5, respectively).[48] The activity of the β-glucosidase having its pH optimum at 4.1 was found to be significantly increased above normal in the diseased brain.

α-Glucosidase activity has been detected on the outside of the labella and legs of the blowfly (*Phormia regina*) and the fleshfly (*Boettcherisca peregrina*).[132] The enzyme activity was not solubilized by the reaction solution during incubation, but remained on the outside of the labella and legs. A large decrease in enzymic activity was observed on prior treatment of the labella and legs with either sodium deoxycholate or sodium dodecyl sulphate. It was suggested that the enzyme activity is located on the membrane of the sensory dendrite at the tip of the chemosensory hair.

One of the two β-galactosidases (pH optima 3.0 and 5.5) obtained from a liver extract of the marine gastropod *Turbo cornutus*, by means of heat and pH-variation treatments, ion-exchange chromatography, and gel filtration, appeared to be a heteroenzyme.[61] The enzyme of pH optimum 5.5 exhibited β-glucosidase, β-galactosidase, β-D-fucosidase, and α-L-arabinosidase activities, in the ratio 5 : 5 : 13 : 1, using 4-nitrophenyl glycosides as substrates. It is regarded as a glycosidase that hydrolyses glycosidic bonds involving terminal, non-reducing residues having the same configuration at C-1, C-2, and C-3 as β-D-galactopyranose. The other enzyme (pH optimum 3.0) was inhibited by cyanide ions, but was resistant to such heavy-metal salts as copper(II) sulphate and mercury(II) chloride. The former enzyme was inhibited by both cyanide and heavy-metal ions, but both enzymes were resistant to the inhibitory actions of sodium azide and H_4edta. Other properties of the enzymes (including their stabilities to heat and pH, and K_m values) were investigated.

Inhibition of the β-glucosidase, β-galactosidase, α-L-arabinosidase, and β-xylosidase activities of sweet almond emulsin by synthetic *C*-glycosides has been investigated.[70] The inhibitory effect of these glycosides was found to decrease in the order 1-β-D-glucopyranosyl-, 1-α-L-arabinosyl-, 1-β-D-galactopyranosyl-, and 1-β-D-xylopyranosyl-4-nitrobenzene; the inhibition was shown to be non-competitive in every case. The evidence indicated that all the inhibitors acted on a single enzyme species.

[132] O. Koizumi, H. Kijima, K. Kawabata, and H. Morita, *Comp. Biochem. Physiol.*, 1973, **44B**, 347.

It has been confirmed that α-glucosidase occurs in the aleurone layer and embryo of ungerminated barley, and that the level of its activity increases during germination.[133] This increase is enhanced by the presence of gibberellin GA_3. Since the activities of α-glucosidase and other enzymes in these tissues have previously been determined as 'α-amylase' by some assay methods, it was pointed out that the response to gibberellin GA_3 necessitates a re-evaluation of the evidence for *de novo* synthesis of α-amylase in aleurone tissues.

The β-glucosidase from germinating seeds of *Dolichos biflorus* has been purified by ion-exchange chromatography of an extract.[57] The enzyme exhibited maximal activity in the pH range 4—5, and its properties during storage were examined.

Changes in the levels of β-glucosidase and various β-glucan hydrolases in the second internode of the stem of developing oat plants have been examined.[134] Concurrent changes in the non-cellulosic β-glucans in the corresponding total hemicelluloses were also noted. Possible relationships between the observed changes and the growth and development of the plant tissue were discussed.

The avoidance of side-effects catalysed by the α-glucosidase impurity in certain preparations of β-amylase from sweet potato by inhibition of the former (with either erythritol or 2-amino-2-hydroxymethylpropane-1,3-diol) has been reported to have some disadvantages.[135] Alternatively, ion-exchange chromatography on DEAE-Sephadex completely separated the two activities.

Rice seeds have been shown to possess two α-glucosidases, whose action on maltose and starch was studied.[136] The activities of both enzymes towards starch were increased 2.3—2.6-fold by the presence of potassium chloride, and activation was also effected by other uni- and bi-valent cations. By contrast, the activities of the enzymes on maltose were not influenced by cations. In experiments with mixed substrates, the liberation of D-[^{14}C]glucose from uniformly labelled [^{14}C]maltose was not inhibited by the presence of starch; moreover, the liberation of D-[^{14}C]glucose from uniformly labelled [^{14}C]starch was not affected by the presence of maltose. From these data it was suggested that the α-glucosidases possess separate sites for the hydrolyses of maltose and starch, and that these sites are regulatory. The enzymes liberated D-glucose only from starch, and are presumed to hydrolyse the polysaccharide completely after prolonged incubation.

Molecular-sieve chromatography of an extract of ungerminated seeds of rye has indicated the presence of enzymes capable of hydrolysing cellobiose, laminaribiose, cellodextrins, laminarin, and barley β-glucan.[137] The enzymes are considered to include a β-glucosidase.

[133] V. J. Clutterbuck and D. E. Briggs, *Phytochemistry*, 1973, **12**, 537.
[134] A. J. Buchala and H. Meier, *Planta*, 1973, **111**, 245.
[135] J. J. Marshall and W. J. Whelan, *Analyt. Biochem.*, 1973, **52**, 642.
[136] N. Takahashi and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 67.
[137] D. J. Manners and J. J. Marshall, *Phytochemistry*, 1973, **12**, 547.

Studies of the cellulolytic enzymes in extracts from the locular contents of ripening tomato (*Lycopersicum esculentum*) fruits showed that cellopentaose, -tetraose, -triose, and -biose, in addition to carboxymethylcellulose, serve as substrates for the production of D-glucose.[138] These properties of the enzyme system are attributed to the presence of a β-glucosidase and a cellulase. The kinetics of both enzymes were investigated, and the β-glucosidase was found to be completely inhibited by D-glucono-1,5-lactone.

β-Glucosidase activity has been recognized in a cellulosic complex of a Basidiomycete species.[139]

The purification, by means of ion-exchange chromatography, gel filtration, and fractionation on hydroxyapatite, of α-glucosidase and β-glucosidase from the spent growth medium of cultures of *Dictyostelium discoideum* myxamoebae has been described.[68] The α-glucosidase was shown to be electrophoretically homogeneous. The pH optima (pH 3.4 and 3.4, respectively), pH and temperature stabilities, kinetic properties, and substrate specificities of the two enzymes were examined. Reaction of either enzyme with concanavalin A produced strong precipitation bands on Ouchterlony plates, and the precipitated form of both enzymes reacted with the corresponding histochemical and fluorogenic substrates. It was concluded that both enzymes contain α-D-mannopyranosyl residues and, possibly, D-glucopyranosyl and 2-acetamido-2-deoxy-D-glucopyranosyl residues.

α-Glucosidase has been obtained from the mycelia of *Mucor javanicus* by procedures that included extraction with urea, fractionations with acetone and poly(ethylene glycol), gel filtration, and ion-exchange chromatography.[140] The enzyme crystallized on the addition of ampholine reagent and, in the purified form, was homogeneous on ultracentrifugation and gel electrophoresis. Various properties (5.6 *S*, p*I* 8.6) of the enzyme were investigated, and it was recognized that the synthesis of riboflavin α-D-glucoside is catalysed by the transglucosylation activity of the enzyme. Further studies related to the transglycosylation action of the enzyme, and substrate and inhibitor specificities have been reported.[141] The α-glucosidase hydrolysed maltose, methyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside, and soluble starch, with the liberation of D-glucose, but was comparatively inactive against phenyl and methyl α-D-glucopyranosides, sucrose, isomaltose, panose, and dextran. Use of a D-glucosyl donor, such as maltose, confirmed the transglucosylation properties of the enzyme, the principal product from maltose being maltotriose; transglucosylation from maltose to riboflavin, pyridoxamine, esculin, and rutin also occurred. 2-Amino-2-hydroxymethylpropane-1,3-diol, H_4edta, 4-chloromercuri-

[138] D. M. Pharr and D. B. Dickenson, *Plant Physiology*, 1973, **51**, 577.
[139] J. Comtat and F. Barnoud, *Compt. rend.*, 1973, **277**, *C*, 61.
[140] Y. Yamasaki, T. Miyake, and Y. Suzuki, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 131.
[141] Y. Yamasaki, T. Miyake, and Y. Suzuki, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 251.

benzoate, and turanose were able to inhibit the enzyme. Inhibition experiments with Rose Bengal and diazonium-1*H*-tetrazole suggested that enzymic activity is closely related to a histidine residue at the active site.

The β-glucosidase involved in the formation of antioxidants (*e.g.* isoflavones) from soybeans fermented with *Rhizopus oligosporus* has been investigated.[142] The crude enzyme, which was obtained by precipitation with ammonium sulphate from an aqueous extract of the organism cultured with a soybean extract, was found to hydrolyse genistin; the reaction mixture from hydrolysis of genistin showed antihaemolytic activity. After further purification by fractional precipitation with acetone, ion-exchange chromatography, and dialysis, the enzyme exhibited maximal activity at pH 5 and 60 °C, and was stable between pH 2 and 9 at 20 °C. K_m Values were determined for various substrates, including genistin, arbutin, and phenyl β-D-glucofuranoside, whereas the enzyme was inactive against methyl α-D-glucopyranoside. Enzymic activity could be masked by inhibition with heavy-metal ions and SH-group reagents, but was reconstituted on treatment with L-cysteine.

Ion-exchange chromatography of the molecular species secreted by protoplasts and intact cells of *Saccharomyces cerevisiae* revealed the presence of three fractions capable of hydrolysing laminarin.[143] The fraction not retained on the column appeared to consist of two enzymes exhibiting activity for laminarin, 4-nitrophenyl β-D-glucopyranoside, and pustulan, and a low activity for periodate-oxidized laminarin. α-Glucosidase has been purified from *S. logos* by precipitation with ethanol, gel filtration, and chromatography on Duolite A-2.[144] The purified enzyme was homogeneous on ultracentrifugation and on zone electrophoresis using a membrane of cellulose acetate. The enzyme possesses the following characteristics: $S_{20,w} = 9.6\,S$, molecular weight 2.7×10^5 (gel filtration), pH optimum 4.6—5.0, and temperature optimum 40 °C; it exhibited hydrolytic activity towards maltose, rather than towards phenyl α-D-glucopyranoside and turanose, and was inactive towards sucrose. As determined by the phenol–sulphuric acid reagent, the enzyme appears to be a glycoprotein containing the equivalent of 50% hexose as D-glucose. Further studies of the substrate activity of the enzyme showed that it is particularly active towards phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside, and the ratio of the rates of hydrolysis of this substrate, maltose, and phenyl α-D-glucopyranoside was 110 : 100 : 5.5.[145] The K_m values for the three substrates in the enzyme system were 3.6, 7.7, and 8.7 mmol l^{-1}, respectively. It was concluded that the substrate specificity of the α-glucosidase is quite different from those of α-glucosidases from

[142] J. Ebeta, Y. Fukuda, K. Hirai, and K. Murata, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 323.
[143] V. Farkaš, P. Biely, and Š, Bauer, *Biochim. Biophys. Acta*, 1973, **321**, 246.
[144] S. Chiba, T. Saeki, and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1823.
[145] S. Chiba, T. Saeki, and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1831.

other *Saccharomyces* species, although its specificity is similar to that of mould α-glucosidases.

The cellulase complex of *Trichoderma koningii* has been separated into four apparently pure components, one of which is a β-glucosidase; the other components were shown to be a cellulase and substances C_1 and C_2.[146] The presence of all four components is necessary for the effective solubilization of cellulose.

β-Glucosidase has been immobilized, with retention of activity, by covalent attachment to cellulose *trans*-2,3-cyclic carbonate[147] and DEAE-cellulose *trans*-2,3-cyclic carbonate.[148]

Glucuronidases

A new, reproducible procedure for the extraction of β-glucuronidase (as a lysosomal glycosidase) from human skin and for assay of the enzyme has been reported.[34] Biopsy specimens from normal skin were frozen, sectioned on a cryostat, and homogenized. The kinetic characteristics of the enzyme were evaluated, and the enzyme was demonstrated to be stable under the conditions employed for the assay. The specific activity of the isolated β-glucuronidase was eight- to ten-fold higher than that previously reported.

β-Glucuronidase activities of homogenates of the fibroblasts derived from normal individuals, from patients affected with disorders associated with a deficiency of α-L-iduronidase (Hurler syndrome, Scheie syndrome, and a Hurler–Scheie compound condition), and from the parents of these patients have been determined, and the values obtained were compared with those of other glycosidases present in the homogenates.[38] Cultured skin fibroblasts derived from a patient with an atypical mucopolysaccharidosis were found to be markedly deficient in β-glucuronidase.[149] The fibroblasts displayed an excessive accumulation and lengthened turnover-times of sulphated glycosaminoglycans. The abnormal metabolism of glycosaminoglycans could be corrected by the addition of bovine β-glucuronidase to the culture medium, whereupon the cells were able to take up the enzyme in amounts far exceeding the requirements for full correction. The identity of the β-glucuronidase and the corrective factor was demonstrated by co-electrophoresis of the two activities on polyacrylamide gels. Skin fibroblasts from patients with I-cell disease have been shown to be deficient in lysosomal β-glucuronidase and α-L-iduronidase, although these enzymes could be detected in the surrounding medium.[37] This situation is not brought about by increased lysosomal leakage, since the I-cells were found to be as retentive towards ingested bovine β-glucuronidase as the cells of other genotypes. Reduced effectiveness in the uptake of the β-glucuronidase from I-cells, compared with the normal enzyme, was found in the correction of β-glucuronidase-deficient cells by the enzyme.

[146] G. Halliwell and M. Riaz, *Arch. Mikrobiol.*, 1971, **78**, 295.
[147] J. F. Kennedy and A. Zamir, *Carbohydrate Res.*, 1973, **29**, 497.
[148] C. J. Gray and T. H. Yeo, *Carbohydrate Res.*, 1973, **27**, 235.
[149] C. W. Hal, M. Cantz, and E. F. Neufeld, *Arch. Biochem. Biophys.*, 1973, **155**, 32.

Two β-glucuronidases and four non-dialysable inhibitors of β-glucuronidase in human pregnancy urine have been separated by ion-exchange chromatography and gel filtration.[150] The inhibitor molecules (molecular weights 2×10^5, 9×10^5, 2×10^3, and 9×10^3) were stable to heat and to the action of trypsin. One of the inhibitors of highest molecular weight was shown to be a glycoprotein containing hexose (20.7), fucose (0.9), 2-amino-2-deoxyhexose (7.4), uronic acid (2.7), and sialic acid (2.0%), together with a trace of sulphate. The action of each inhibitor was non-competitive and was completely suppressed by sodium sulphate.

Purification (by affinity chromatography) of the β-glucuronidase from various normal and tumour tissues and from bovine liver has been described.[151] The column was prepared from a derivative of Sepharose to which either 2-aminophenyl β-D-glucopyranuronoside or saccharo-1,4-lactone had been covalently attached. In the latter case, a spacer molecule was incorporated between the polysaccharide and the monosaccharide, and the composite material was found to retain the enzymic activity more effectively.

The activity and intracellular distribution of several glycosidases (including β-glucuronidase) in rabbit liver have been examined.[47] Glycosides of the steroid oestrogen, which have been shown to occur naturally in rabbits, were used as substrates; a comparison of these results with those obtained for synthetic 4-nitrophenyl glycosides revealed identical distributions of the enzymes at the intracellular level. The β-glucuronidase was found to be concentrated in the lysosomes and showed a latency typical of lysosomal acid glycohydrolases. Intravenous injection of an iron–dextran preparation (Imferon) was followed by a dramatic increase in the β-glucuronidase activity in the lymph nodes of both male and female rabbits.[152] The observations are indicative of a basic role of β-glucuronidase in the phagocytic process.

The activity of a β-glucuronidase (pH optimum 4.5) in normal and scrapie-affected mouse brain has been investigated.[48] The activity of the enzyme in the diseased brain is considerably greater than normal, and the level of enzymic activity had to be greater than normal before the host displayed signs of the disease. Changes in the concentrations of β-glucuronidase and β-galactosidase during the development of mouse brain, liver, and heart were found to be co-ordinated.[103] Although co-ordinated, the developmental patterns of the two enzymes are under independent control by elements apparently linked to the respective structural genes.

A study has been made of the purification of certain glycosidases from rat liver by affinity chromatography on derivatized Sepharose matrices to

[150] W. Sakomoto and O. Nishikaze, *Enzyme*, 1972, **13**, 211.
[151] R. G. Harris, J. J. M. Rowe, P. S. Stewart, and D. C. Williams, *F.E.B.S. Letters*, 1973, **29**, 189.
[152] B. Ballantyne and P. J. Guillou, *Microbios*, 1973, **7**, 181.

which 4-aminophenyl glycosides had been attached covalently *via* their free amino-groups.[52] The β-glucuronidase and corresponding glycosidases were retained by matrices to which 4-aminophenyl glycosides of β-D-glucopyranosyluronic acid, β-D-galactopyranose, or 2-acetamido-2-deoxy-β-D-glucopyranose had been attached. The β-glucuronidase could be eluted from the columns by solutions of either substrate or inhibitor or sodium chloride. The β-glucuronidase activity was separated from β-acetamidodeoxyglucosidase activity using a matrix containing attached 4-aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and step-wise gradient elution with sodium chloride solutions. The purification of β-glucuronidase from an extract of rat-liver lysosomes by fractional precipitation with ammonium sulphate, treatment with trypsin, gel filtration, and ion-exchange chromatography yielded five active fractions.[153] All five isoenzymes were shown to be electrophoretically and ultracentrifugally homogeneous ($S_{20,w}$ = 12.3—13.4) and, since they were found to contain up to 5% carbohydrate, are glycoproteinaceous in nature.

Rat-liver microsomes, free from lysosomal β-glucuronidase, liberated 95% of the microsomal β-glucuronidase, but only 11% of the albumin, in soluble forms on sonication.[154] The results indicate that the enzyme is not contained in the cisternae of the microsomal vesicles, but is attached to the membrane by a bond that is broken by sonication before the membrane is disrupted. The distribution of lysosomal β-glucuronidase between parenchymal and Kupffer cells of rat liver has been investigated.[155]

A search for β-glucuronidase, by immunodiffusion methods combined with staining reactions, in a plasma-membrane fraction isolated from the 4-dimethylaminoazobenzene-induced rat hepatoma D23 revealed the presence of the enzyme.[53] The enzymically active antigen was also found in tumour microsomes, with a varied distribution.

The β-glucuronidase activities of different parts of the male reproductive tract of the giant octopus (*Octopus dofleini martini*) have been determined; the spermatophore and the 'prostate' were shown to be the major sources of the enzyme.[54]

The hydrolyses of urinary steroidal conjugates by intracellular β-glucuronidase from *Escherichia coli* producing high levels of the enzyme have been investigated.[156] The enzyme was found to hydrolyse quantitatively different classes of steroidal D-glucuronides at high rates.

Iduronidases

Using phenyl α-L-idopyranuronoside as substrate, the levels of α-L-iduronidase activity in homogenates of the fibroblasts derived from normal individuals, from patients affected with disorders involving a deficiency of

[153] M. Potier and R. Gianetto, *Canad. J. Biochem.*, 1973, **51**, 973.
[154] L. Mameli and R. Gianetto, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 316.
[155] T. Berg and D. Boman, *Biochim. Biophys. Acta*, 1973, **321**, 585.
[156] J. Dray, F. Tillier, F. Dray, and A. Ullmann, *Ann. Inst. Pasteur*, 1972, **123**, 853.

α-L-iduronidase (Hurler syndrome, Scheie syndrome, and a disease of intermediate severity presumed to be a Hurler–Scheie compound), and from the parents of these patients have been determined.[38] The extracts derived from the affected individuals had no detectable α-L-iduronidase activity, whereas those derived from heterozygotes varied between 20 and 95% of the mean value for normal individuals. The overlap between the normal and heterozygote levels was reduced when the α-L-iduronidase activity was expressed in relation to the β-galactosidase activity of the same homogenate. Cultured amniotic fluid cells from normal pregnancies had less than half as much α-L-iduronidase activity as fibroblasts from normal adults, and this might cause problems in distinguishing between heterozygous and affected foetuses by means of enzymic assay alone.

Skin fibroblasts derived from patients with I-cell disease were found to be deficient in lysosomal α-L-iduronidase and β-glucuronidase activities, although the enzymes are present in the surrounding medium.[37] This situation is considered not to have been brought about by increased lysosomal leakage, since I-cells were found to retain ingested human urinary α-L-iduronidase as effectively as cells of other genotypes. Compared with the normal enzyme, a reduced effectiveness in the uptake of the enzyme from the I-cell medium in the correction of Hurler cells by α-L-iduronidase was observed.

Mannosidases

1-Aminoglycosides have been reported to represent a new class of specific and relatively potent inhibitors of glycosidases, and were found to be specific against enzymes that act upon glycosides corresponding to the glycone of the inhibitor.[23] Thus, α-mannosidase from porcine kidney was inhibited by β-D-mannopyranosylamine, but by neither α-D-galactopyranosylamine nor β-D-glucopyranosylamine.

Electrophoresis, on cellulose acetate, of extracts of human tissues has demonstrated the presence of three forms of α-mannosidase activity, which were identified by treating the matrix with 4-methylumbelliferyl α-D-mannopyranoside to produce fluorescent spots.[157] One band stained with the substrate at a neutral pH, whereas the other two bands were stained at an acid pH. The distribution of the three forms in human brain, fibroblasts, kidney, liver, and placenta was assessed; all were found to be present in these tissues, except that the 'neutral' form was not detected in fibroblasts. The 'acid' and 'neutral' α-mannosidases exhibited pH optima of 3.5—4.0 and 6.5—7.0, respectively.

A new, reproducible method for the extraction of β-mannosidase (as a lysosomal glycosidase) from human skin and for assay of the enzyme has been reported.[34] Biopsy specimens from normal skin were frozen, sectioned on a cryostat, and homogenized. The enzyme was demonstrated to be stable

[157] L. Poenaru and J.-C. Dreyfus, *Biochim. Biophys. Acta*, 1973, **303**, 171.

under the assay conditions and its kinetic characteristics were determined. Using these techniques, the specific activity of the enzyme was found to be eight- to ten-fold higher than that previously reported.

The β-mannosidase activity of human synovial fluid has been assayed.[158] Using 4-nitrophenyl β-D-mannopyranoside as substrate, the enzyme was found to possess a K_m value of 3.4 mmol l^{-1} and a pH requirement of 3.5—4.0. The β-mannosidase is significantly more labile to heat than mammalian α-mannosidase. 4-Nitrophenyl β-D-galactopyranoside inhibited the β-mannosidase, and competition studies led to the conclusion that the enzyme is an independent glycosidase. The β-mannosidase was demonstrated to be widely distributed in mammalian tissues and to occur in human lymphocytes, fibroblasts, polymorphonuclear cells, synovia, and sera, and in the brain, lung, liver, spleen, kidney, and muscle of mice. The enzyme was also detected in human synovia from cases of rheumatoid arthritis and pigmented villous nodular synovitis.

α-Mannosidase has been purified from an extract of porcine kidney until it was homogeneous on disc electrophoresis; in the presence of sodium dodecyl sulphate, a molecular weight of 4.2×10^4 was obtained.[159] The enzyme exhibits a broad specificity for aglycones and is capable of hydrolysing α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 6)-linked oligomannosides, *O*-α-D-mannopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-glucose, *O*-α-D-mannopyranosyl-(1 → 6)-2-acetamido-2-deoxy-D-glucose, and the α-linkage of *O*-α-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose. The magnitudes of the values of K_m and V_{max} were found to depend on the structures of the oligosaccharide substrates. The α-mannosidase was shown to be a glycoprotein containing mannose (8) and 2-amino-2-deoxyglucose (3.3%).

The effects of metal ions upon the 'acid' and 'neutral' α-mannosidases in an extract of rabbit liver have been investigated.[102] Whereas the activity of the 'acid' form was inhibited by Cd^{2+} ions, that of the 'neutral' form was increased by 420% by these ions; the augmented activity remained after gel filtration of the treated enzyme. Similar activation was observed for the 'neutral' enzyme in homogenates of the liver, spleen, and epididymis of rabbits and rats, and of rat kidney. By contrast, the 'acid' enzyme from rabbit liver was activated by Zn^{2+} ions, whereas the 'neutral' enzyme was unaffected. Treatment with Zn^{2+} ions also restored the activity of the 'acid' enzyme that is inhibited by H_4edta. The different responses to bivalent metal ions provide evidence that the two α-mannosidases are distinct species.

The pH optimum of the α-mannosidase in normal and scrapie-affected mouse brain has been investigated and, over the range examined, the enzyme displayed two peaks of activity (pH 4.1 and 6.0).[48] The level of α-mannosidase activity in the diseased brain was shown to be significantly

[158] B. A. Bartholomew and A. L. Perry, *Biochim. Biophys. Acta*, 1973, **315**, 123.
[159] T. Okumura and I. Yamashina, *J. Biochem. (Japan)*, 1973, **73**, 131.

greater than normal, and the level of one of the enzymes (pH optimum 4.1) is elevated before the mice exhibit signs of the onset of the condition. β-Mannosidase activity has been located in the lysosomes of rat liver.[51] The enzyme was distinguishable from the α-mannosidase, possessed optimum activity at an acid pH, and was stable at pH values up to 10. Purified lysosomal preparations hydrolysed the trisaccharide *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose at rates proportional to those at which 4-nitrophenyl β-D-mannopyranoside was hydrolysed. Further studies of the substrate specificity of the enzyme showed that it is capable of hydrolysing the trisaccharide at a faster rate than the corresponding disaccharide, and that it cleaves the carbohydrate moieties of glycopeptides obtained from ovalbumin. The subcellular distribution of the α-mannosidase was also investigated.

The activities of various glycosidases, including α-mannosidase, in different parts of the male reproductive tract of the giant octopus (*Octopus dofleini martini*) have been investigated.[54] The spermatophore and the 'prostate' were shown to be major sources of the enzymes, with the α-mannosidase concentrated in the cement liquid.

The purification and the ion-exchange chromatographic properties of the α-mannosidase of germinating seeds of *Dolichos biflorus* have been reported.[57] The enzyme was shown to exhibit maximum activity at pH 4.0.

The α-mannosidase from an extract of Jack-bean meal has been purified by precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[58] Ion-exchange chromatography separated the α-mannosidase from a β-acetamidodeoxyglucosidase present in the extract.

The levels of α- and β-mannosidase and β-galactosidase activities in extracts from the endosperm and embryo of fenugreek (*Trigonella foenum-graecum*) seeds have been determined at various stages of germination.[106] The onset of breakdown of galactomannan coincided with the appearance of α-galactosidase and β-mannosidase activities, which were found to increase throughout the period of galactomannan degradation, whereafter they remained constant.

As well as being a glycohydrolase, the α-mannosidase from seeds of *Vicia sativa* was able to transfer D-mannosyl residues from phenyl α-D-mannopyranoside to ketoses and pentoses.[160] With this particular donor and D-ribose as acceptor, three isomeric D-mannosides were formed; on the basis of methylation analyses, the structures 2-, 3-, and 5-*O*-α-D-mannopyranosyl-D-ribose were proposed for these products.

The isolation and characterization of α-mannosidase from baker's yeast (*Saccharomyces* sp.) have been described.[161] After purification on hydroxyapatite, the enzyme (pH optimum 6.8), was found to hydrolyse phenyl and 4-nitrophenyl α-D-mannopyranosides but to release only trace amounts of D-mannose from intact D-mannan. On gel filtration, the enzyme

[160] A. Zurowska, E. Vilarroya, and F. Petek, *Carbohydrate Res.*, 1972, **24**, 319.
[161] T. Kaya, M. Shibano, and T. Kutsumi, *J. Biochem. (Japan)*, 1973, **73**, 181.

displayed a molecular weight of 3×10^5, and the properties of the α-mannosidase were compared with those of other α-mannosidases.

The purification of α-mannosidase and other glycosidases from the spent growth medium of *Dictyostelium discoideum*, by means of ion-exchange chromatography, gel filtration, and column chromatography on hydroxyapatite, has been described.[68] The final preparation exhibited maximum activity at pH 3.0, but the pH optimum was very broad, and two distinct isoenzyme bands were observed on electrophoresis. The pH optima, stabilities to pH and temperature, kinetic properties, and substrate specificity of the α-mannosidase were examined. Reaction of the glycosidases with concanavalin A produced strong bands on Ouchterlony plates in each case, and the precipitated concanavalin A–enzyme complexes reacted with their corresponding histochemical and fluorogenic substrates. It was concluded that each enzyme contains α-D-mannopyranosyl and, possibly, D-glucosyl and 2-acetamido-2-deoxy-D-glucopyranosyl residues.

Growth of the fungus *Penicillium charlesii* on a medium of D-glucose and minimal concentrations of salts resulted in the appearance of α-mannosidase activity capable of hydrolysing 4-nitrophenyl α-D-mannopyranoside.[162] However, activity could not be detected until the source of carbon became depleted, whereafter the α-mannosidase activity of the mycelia increased rapidly. Prolonged incubation of the culture resulted in the appearance of small amounts of the enzyme in the growth medium. The initial release of a D-mannose-containing polysaccharide into the medium preceded the appearance of α-mannosidase activity by several days.

The patterns of intra- and extra-cellular α-mannosidase activity during microcrystalline differentiation have been studied in the cellular slime-mould *Polysphondylium pallidum*.[163] α-Mannosidase activity was present in amoebae and increased with differentiation; further information indicated that the increase in enzyme activity requires concurrent synthesis of protein. A cyst-less mutant of the organism did not exhibit the normal intracellular pattern of the α-mannosidase, although it did excrete the enzyme.

Rhamnosidases

In studies of the actions of zosterinases from the surface snails *Eulota maackii* and *Helix pomatia* on zosterine, it was found that both enzymes liberated trace amounts of L-rhamnose from the substrate.[164]

α-L-Rhamnosidase activity has been detected in the liver of a marine gastropod (*Turbo cornutus*) using 4-nitrophenyl α-L-rhamnopyranoside as substrate.[165] The enzyme was purified by means of ion-exchange

[162] J. F. Preston, E. Lapis, and J. E. Gander, *Arkiv. Mikrobiol.*, 1973, **88**, 71.
[163] D. H. O'Day, *J. Bacteriol.*, 1973, **113**, 192.
[164] V. I. Shibaeva, R. G. Ovodova, and Y. S. Ovodov, *Comp. Biochem. Physiol.*, 1973, **46B**, 561.
[165] Y. Kurosawa, K. Ikeda, and F. Egami, *J. Biochem. (Japan)*, 1973, **73**, 31.

chromatography, gel filtration, heat treatment, and freezing and thawing in an acid medium. α-L-Rhamnosidase was also purified from 'Naringinase', a commercial product prepared from *Aspergillus niger*. The two enzymes possessed somewhat different properties and specificities. The enzyme from *T. cornutus* (pH optimum 2.8) readily hydrolysed quercitrin (quercitin-3-α-L-rhamnopyranoside), but not naringin [naringenin-7-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)]. On the other hand, the α-L-rhamnosidase (pH optimum 4.5—5.0) from *A. niger* readily hydrolysed naringin and rutin [quercitin-3-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside)], but not quercitrin. Neither methyl α-L-rhamnopyranoside nor rhamnosyl 2-keto-3-deoxyoctonate could be hydrolysed by these enzymes. The effects of various substances on the activities of enzymes were studied, but no specific inhibitors were found. Unlike the crude preparation of mixed glycosidases from the liver of *T. cornutus*, the purified α-L-rhamnosidase did not release L-rhamnose from a lipopolysaccharide of *Pseudomonas aeruginosa*.

α-L-Rhamnosidase activity has been detected in a culture filtrate from *Corticium rolfsii* using 4-nitrophenyl α-L-rhamnopyranoside as substrate;[166] the enzyme possessed a remarkably low pH optimum (2—3) and was most stable in the pH range 2—6. The curves for the activity and stability are non-symmetrical, being much steeper on the side of lower pH.

Sialidases

The effects of intravenous neuraminidase on the turnover of mammalian fibrinogen have been discussed.[167]

The subcellular distribution of the major particulate neuraminidase activity in rabbit cortex has been determined.[168] The activity was present in the nerve endings (40%), the light membrane of microsomal origin (50%), and a myelin-rich preparation obtained from a crude mitochondrial fraction (8%). The ganglioside distribution followed this pattern both qualitatively and quantitatively. Conversely, other cell preparations were devoid of both neuraminidase and gangliosides. The results are consistent with the hypothesis that neuraminidase and gangliosides together are fundamental components of the neuronal plasma membrane.

A method for the purification of neuraminidase from the heart muscles of rats has been described.[26] The highly purified enzyme was found to possess activity towards both mono- and poly-sialogangliosides and fetuin, but not towards *O*-*N*-acetyl-α-neuraminosyl-(2 → ?)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose. The enzyme exhibited maximum activity at pH 5.3, and the K_m values for various ganglioside substrates were determined. Studies of the kinetic parameters of the enzyme were performed,

[166] A. Kaji and T. Ichimi, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 431.
[167] E. Regoeczi and K.-L. Wong in 'Protein Turnover', ed. G. E. W. Wolstenholme and M. O'Connor, Associated Scientific Publishers, Amsterdam, 1973, p. 181.
[168] G. Tettamanti, A. Preti, A. Lombardo, F. Bonali, and V. Zambotti, *Biochim. Biophys. Acta*, 1973, **306**, 466.

and ammonium sulphate, Cu^{2+} and Fe^{2+} ions, 4-chloromercuribenzoate, and a derivative of dihydroisoquinoline acted as inhibitors. A scheme for the total hydrolysis of ganglioside-$G_{T1a/b}$ and related gangliosides was presented.

Neuraminidase from *Clostridium welchii* has been purified by means of both affinity and ion-exchange chromatography.[169] Loss of activity resulted either from oxidation of the tryptophanyl residues of the enzyme with *N*-bromosuccinimide or by modification of the enzyme with 2-hydroxy-5-nitrobenzyl bromide. It was concluded that the tryptophanyl residues play an essential role in maintaining the structure of the protein molecule necessary for the manifestation of activity. A derivative of Sepharose, to which *N*-(4-aminophenyl)oxamic acid groups had been covalently attached, has also been used for purification of the neuraminidase from *C. welchii* by affinity chromatography.[170] At pH 5.5, the enzyme was quantitatively adsorbed by, and eluted from, the column. Since this enzyme is one of a number of extracellular proteins produced by *C. welchii*, the specificity of the method was tested by following the passage of several proteins through the affinity column. It was found that haemagglutinin, haemolysin, and phospholipase C behave in the same way as neuraminidase, insofar as they were adsorbed on to, and quantitatively eluted from, the column at pH 9.1. Further studies showed that all of these proteins (including neuraminidase) are adsorbed on to the Sepharose-glycyltyrosine backbone of the affinant, but not on to Sepharose itself. When these proteins were applied to a column of Sepharose-glycyltyrosine at pH 7.5, the non-specific adsorption was largely overcome: haemagglutinin and haemolysin passed straight through the column, whereas sialidase was weakly adsorbed and was leached off slowly with continuous washing. These results showed that sialidase is bound more firmly to the affinity matrix than to Sepharose-glycyltyrosine, but they do not indicate whether this effect is specific or non-specific. The action of commercial preparations of neuraminidase from *C. welchii* on mixed gangliosides G_{M1} and G_{M2} from bovine brain has been investigated in the presence of various bile salts.[171] The ability of the enzyme to produce the corresponding asialo-derivatives in these circumstances is considered to provide a new means of investigating the structures of gangliosides and the metabolism of glycolipids.

A survey of representative Corynebacteria (plant pathogenic Corynebacteria, Mycobacteria, and Nocardias) revealed that only *Corynebacterium diphtheriae* and closely related Corynebacteria exhibit neuraminidase and *N*-acetylneuraminate lyase activities.[172] A method has been described for the isolation of neuraminidase from a non-toxicogenic strain of

[169] H. Bachmayer, *F.E.B.S. Letters*, 1972, **23**, 217.
[170] J. I. Rood and R. G. Wilkinson, *Proc. Austral. Biochem. Soc.*, 1973, **6**, 9.
[171] D. A. Wenger and S. Wardell, *J. Neurochem.*, 1973, **20**, 607.
[172] S. B. Arden, W. H. Chang, and L. Barksdale, *J. Bacteriol.*, 1972, **112**, 1206.

C. diphtheriae by its precipitation from the culture fluid with zinc chloride and repeated fractionation by gel filtration.[173] The method yielded an enzyme preparation that was electrophoretically and antigenically homogeneous.

The effect of neuraminidase from *Diplococcus pneumoniae* on canine cerebral cortex in experimental meningitis has been studied.[174]

Xylosidases

Application of a computer to the automation of a recording polarimeter for the assay of β-xylosidase activity has been described (see p. 358).[18]

Investigation of the β-xylosidase activity in normal and scrapie-affected brains of mice showed that the enzyme exhibits maximum activity at pH 4.5.[48] The β-xylosidase and other glycosidase activities of the diseased brain were found to be considerably greater than normal.

In a study of the action of zosterinases from the surface snails *Eulota maackii* and *Helix pomatia* on zosterine, both systems were found to release trace amounts of D-xylose from the substrate.[164]

Inhibition of the β-xylosidase, β-glucosidase, β-galactosidase, and α-L-arabinosidase activities of sweet almond emulsin by synthetic *C*-glycosides has been examined.[70] The inhibitory effects of the *C*-glycosides were found to decrease in the order 1-β-D-glucopyranosyl-, 1-α-L-arabinosyl-, 1-β-D-galactopyranosyl-, and 1-β-D-xylopyranosyl-4-nitrobenzene. The inhibition was shown to be non-competitive in every case, and the results suggested that all the *C*-glycosides act on the same species of enzyme.

The β-xylosidase of *Aspergillus niger* has been extracted from a culture of the organism on wheat-bran koji and was purified by way of ion-exchange chromatography, gel filtration, and column electrophoresis.[175] The purified enzyme, which was practically free from xylanase and other glycosidase activities, was most active at pH 3—4, and was most stable in the pH range 4—7. The β-xylosidase exhibited glycosyltransferase activity and synthesized *O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose from xylobiose and D-xylose, and the corresponding xylotetraose and xylopentaose from xylotriose. It was concluded that the enzyme is capable of transferring a D-xylosyl unit preferentially to O-4 of the appropriate xylo-oligosaccharides. This behaviour is characteristic of the β-xylosidase from this particular mould, since sugar residues are generally transferred to a number of positions on the acceptors in the transglycosylation processes of other glycosidases.

endo-α-Acetamidodeoxygalactosidase

The action of an enzyme from culture filtrates of *Clostridium welchii* (*perfringens*) on porcine submaxillary glycoprotein resulted in the release

[173] Y. V. Vertiev and Y. V. Ezepchuk, *Folia Microbiol.*, 1972, **17**, 269.
[174] M. M. Carruthers and R. Kanokvechayant, *Infection and Immunity*, 1973, **7**, 370.
[175] S. Takenishi, Y. Tsujisaka, and J. Fukumoto, *J. Biochem.* (*Japan*), 1973, **73**, 335.

of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose.[67] The possibility that this disaccharide resulted from transglycosylation was excluded. The same disaccharide was obtained by the action of the enzyme on a number of other glycoproteins and the blood-group substances. Since it is known that 2-acetamido-2-deoxy-D-galactopyranosyl residues at the reducing ends of oligosaccharide chains of the blood-group substances are α-linked to hydroxyamino-acids of the protein core, the enzyme is considered to be an *endo*-α-acetamidodeoxygalactosidase. This enzyme could prove useful in structural studies of glycoproteins.

Agarases

An enzyme that cleaves the β-(1 → 4), rather than the β-(1 → 3), glycosidic linkages in agarose has been identified in a *Bacillus* species.[176] Products resulting from the action of the enzyme on the polysaccharide were shown to contain agarose oligosaccharides.

Alginases

The alginase contained in an aqueous extract of the hepatopancreas of a mollusc (*Littorina* sp.) has been purified by fractional precipitation with ammonium sulphate, gel filtration, and affinity chromatography.[177] For the latter procedure, a matrix was produced by treatment of Bio-Gel P-20 with hydrazine hydrate, followed by 4-dimethylaminobenzaldehyde; the adsorbed enzyme was subsequently desorbed with buffered saline. Throughout the purification, alginase activity was monitored by determination of the reducing power generated from an alginate substrate; disc electrophoresis of the final enzyme preparation indicated that it was substantially homogeneous.

Alginate Lyases

Evidence has been obtained for the occurrence of an alginate-degrading enzyme system in peripheral tissues of the brown alga (*Laminaria digitata*).[178] A partially purified preparation of the alga effected rapid reduction of the viscosity of solutions of sodium alginate, with a concomitant rise in material giving a positive response in a Warren (2-thiobarbituric acid) assay. An alginate lyase was also prepared from a particulate fraction of the interior layers of the alga.

α-Amylases

A coloured derivative of limit dextrin, obtained by treating commercial Amylopectin Azure with β-amylase, has been used as the basis of a new and simple method for differentiating between α- and β-amylases.[179] The

[176] M. A. Vattuone and A. R. Sampietro, *Compt. rend.*, 1973, **276**, *D*, 3225.
[177] V. V. Favorov, *Internat. J. Biochem.*, 1973, **4**, 107.
[178] J. Madgwick, A. Haug, and B. Larsen, *Acta Chem. Scand.*, 1973, **27**, 711.
[179] D. E. Bilderback, *Plant Physiol.*, 1973, **51**, 594.

dye-limit dextrin was demonstrated to be a substrate entirely specific for α-amylase, and the presence of β-amylase in the α-amylase under test had little effect on the release of carbohydrate–dye complexes.

A Blue Starch method, involving the use of a Phadebas Amylase test, has been used for the determination of amylase activity in serum and urine samples.[180] The method was found to be simple and reproducible, and the colour released into solution by the reaction is very stable, and is not affected by the presence of haemoglobin, bilirubin, or sugars.

A method has been described for the purification of α-amylase from human pancreatic juice by means of fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[181] A product having a high degree of purity was obtained in 59% yield, and its assessment on polyacrylamide-gel electrophoresis revealed a characteristic pattern of isoenzymes, with up to six bands being recognized on anionic gels. Comparisons of the enzyme with crystalline, human-parotid α-amylase indicated that the two are closely related, but that they also exhibit organ-specific variations. Each enzyme appeared to consist of a single polypeptide chain, which yielded the same amino-acids and similar, but not identical, peptide maps on hydrolysis. At neutral pH, both α-amylases exhibited the same action pattern, but differences were noted in the gel-filtration profiles, the molecular weights (pancreatic 5.4×10^4; parotid 5.6×10^4 and 6.2×10^4 according to its source, *viz.* A and B family of isoenzymes, respectively), the carbohydrate contents, and the accessibilities of reactive SH groups. At both high- and low-temperature extrema, the pancreatic enzyme was comparatively more labile than the parotid enzyme, and a less compact configuration was indicated for the pancreatic α-amylase. A relation between the number of SH groups in the α-amylase molecule and its mode of action was suggested. α-Amylases possessing one SH group per molecule, namely those from human pancreatic juice and parotid saliva and from rat parotid saliva, exhibited the same action pattern, which was different from that of pancreatic α-amylases possessing two SH groups per molecule.

Human urinary α-amylase has been crystallized.[182] The enzyme gave a single band on electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulphate, but four isoenzymes were distinguished on electrophoresis on cellulose acetate. Two of these forms were electrophoretically indistinguishable from two of the three isoenzymes of α-amylase from human pancreas. The other two urinary forms were comparable with two of the three isoenzymes of salivary α-amylase. The action patterns of the urinary, crystalline salivary, and pancreatic enzymes were compared by paper chromatography of the products resulting from

[180] A. Irie, M. Hunaki, K. Bando, and K. Kawai, *Clinica Chim. Acta*, 1972, **42**, 63.
[181] D. J. Stiefel and P. J. Keller, *Biochim. Biophys. Acta*, 1973, **302**, 345.
[182] N. Minamiura, K. Umeki, K. Tsujino, and T. Yamamoto, *J. Biochem.* (*Japan*), 1972, **72**, 1295.

their action on β-limit dextrin. The action pattern of the urinary enzyme was quite similar to that of each of the other two, although it differed considerably from that of α-amylases from other sources in the sizes of the oligosaccharides liberated. After removal of the α-amylase, urine from humans was found to contain a residual glucanase that converted starch into D-glucose.

Parotid amylase from the baboon (*Papio anubis*) has been obtained in crystalline form.[183] Examination, by disc electrophoresis, of the crystalline material and the original material fractionally precipitated by ammonium sulphate revealed the presence of three amylolytic species. In contrast to isoenzymes from human parotid amylase, which can be separated into two classes (glycoenzymes and non-glycoenzymes) during re-cycling on gel-filtration columns, the isoenzymes from baboon parotid amylase emerged as one peak from these columns. On disc-gel electrophoresis in the presence of sodium dodecyl sulphate, the isoenzymes from baboon amylase migrated as a single band. The molecular weight of the species was estimated to be 5.29×10^4, compared with a value of 5.6×10^4 for the analogous species from human parotid amylase. The enzymes from baboon did not appear to be glycoenzymes, as evidenced by a negative reaction for neutral carbohydrate.

Biliary amylases from bovine and ovine sources have been studied.[184]

α-Amylase has been purified from an extract of porcine pancreas by selective adsorption on to glycogen, followed by desorption and crystallization from a concentrated solution in the presence of Ca^{2+} ions.[185] Two morphological forms of the crystals were recognized, and *X*-ray analysis showed them to be of identical space grouping ($a = 7.0$, $b = 11.0$, $c = 11.7$ nm). The diffraction pattern gave a resolution of better than 0.3 nm, and the crystals appeared to be stable to the radiation used. The asymmetric unit consists of two molecules having a total molecular weight of 9.0×10^4. Porcine pancreatic α-amylase prepared from mammalian sources on a commercial basis is contaminated with ribonuclease, which may be removed completely by treatment of the enzyme solution with bentonite.[186]

Porcine pancreatic α-amylase has been shown to be a glycoprotein; both molecular forms (I and II) of the enzyme contain galactose (0.45, 0.45), mannose (0.52, 0.52), fucose (0.75, 0.75), and 2-amino-2-deoxyglucose (1.2 and 0.8 moles per mole, respectively).[187] Investigation of the neutral carbohydrates liberated from the enzyme with 0.1M-H_2SO_4 showed that fucose is released most readily.

[183] B. L. Williams and P. J. Keller, *Comp. Biochem. Physiol.*, 1973, **44A**, 393.
[184] P. Ceccarelli, A. Debenedetti, and A. Lucaroni, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 539.
[185] A. McPherson and A. Rich, *Biochim. Biophys. Acta*, 1972, **285**, 493.
[186] C. T. Garrett, D. S. Wilkinson, and H. C. Pitot, *Analyt. Biochem.*, 1973, **52**, 342.
[187] B. Beaupoil-Abadie, M. Raffalli, P. Cozzone, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1973, **297**, 436.

The action of porcine pancreatic α-amylase on glycogen has been investigated.[188] Apo-α-amylases from both porcine pancreas and *Bacillus subtilis* have been prepared by continuous dialysis of the enzymes against H_4edta in a Colowick cell to remove the tightly-bound calcium ions.[189] In both cases, introduction of lanthanide ions failed to reactivate the enzymes. The rare-earth metal ions did not compete for the sites binding calcium ions and, therefore, did not inhibit reactivation of the enzymes with calcium ions. The *B. subtilis* α-amylase was shown to possess two sites for binding calcium ions, one of which could bind gadolinium(III) ions, and an additional site for binding lanthanide ions.

The effect of maltose on the structure of porcine pancreatic α-amylase has been studied.[190] The difference spectrum obtained was characteristic for the perturbation of tryptophanyl residues and appeared to be specific for maltose. One tryptophanyl residue per molecular chain of the enzyme was indicated to be involved in the interaction. The dissociation constant of the α-amylase–maltose complex was calculated from the dependence of the difference absorption spectrum on the concentration of maltose. The activity of the enzyme was completely inhibited in the presence of maltose, and the inhibition constant was determined. The results imply that interaction of the tryptophanyl side-chain with maltosyl residues may be involved in the binding of the α-amylase to substrates. Two masked SH groups of porcine pancreatic α-amylase have been shown to react with 5,5′-dithiobis-(2-nitrobenzoate), and first-order kinetics were followed in the presence of H_4edta.[191] The correlation between the reactivity of these SH groups and the conformational mobility of the enzyme was studied. Neither oxidation nor formation of the mixed (enzyme-2-nitro-5-mercaptobenzoate) disulphide influenced the activity of the enzyme, but both treatments resulted in decreased stability.

The 'neutral' α-glucosidase activity of rabbit muscle was shown to possess constitutive 1,4- and 1,6-α-glucanohydrolase activities, although it possessed little action on glycogen, which it is considered to hydrolyse in conjunction with α-amylase.[131] The combined action of these enzymes is thought to have an ancillary role in controlling the structure of glycogen.

The distribution of α-amylase activity among the various subcellular and submicrosomal fractions of homogenates obtained from the livers from normally fed and fasted rats has been investigated by differential and density-gradient centrifugation.[192] The major part of the α-amylase activity was found in rough and smooth microsomal fractions. A portion of this activity was adsorbed by the microsomes, but was released on sonication, thus supporting the view that liver amylase is a secretory

[188] G. L. Brammer, M. A. Rougvie, and D. French, *Carbohydrate Res.*, 1972, **24**, 343.
[189] A. Levitzki and J. Reuben, *Biochemistry*, 1973, **12**, 41.
[190] P. Elödi, S. Móra, and M. Krysteva, *European J. Biochem.*, 1972, **24**, 577.
[191] M. Telegdi and F. B. Straub, *Biochim. Biophys. Acta*, 1973, **321**, 210.
[192] K. Hammerton and M. Messer, *Biochim. Biophys. Acta*, 1973, **321**, 597.

protein. The total α-amylase activity and that associated with glycogen were reduced in fasting animals, whereas the activity of the enzyme in the postmicrosomal supernatant was increased.

Intact aleurone layers of barley (*Hordeum distochon*), on incubation under optimum conditions with gibberellin GA_3, produced near-maximal amounts of α-amylase; the enzyme accumulated in the tissues before its release into the medium.[133] However, daily replacement of the incubation medium (containing gibberellin GA_3) depressed the quantity of α-amylase produced. The α-amylase was also produced in response to gibberellins GA_1, GA_4, and GA_7, and, to a much lesser extent, helminthosporol and helminthosporic acid. A range of other substances previously reported to induce the formation of α-amylase failed to do so in these experiments. It was confirmed that α-glucosidase occurs in the aleurone layer and the embryo of ungerminated barley, and that its level increases during germination; gibberellin GA_3 was shown to enhance this increase. Since the activities of α-glucosidase and other enzymes have previously been determined as 'α-amylase' by some assay methods, it is considered that the alterations of enzymic activity in response to gibberellin GA_3 necessitate a re-evaluation of the evidence for *de novo* synthesis of α-amylase in aleurone tissue. The scutellar tissue of germinating barley (*H. distochon*) is able to synthesize significant amounts of α-amylase.[193] The endogenous levels of sugars are considered to restrict the initial formation of α-amylase, principally by repressing the supply of gibberellins to the aleurone layer.

α-Amylases of the endosperm of maize, oat, and wheat seeds have been investigated by immunodiffusion, electrophoresis, and immunoelectrophoresis after five to six days of germination.[194] The α-amylase activity was found under a single molecular form in wheat seeds, and under two and four molecular forms in maize and oat seeds, respectively. Antigenically, the enzymes were found to be species-specific. α-Amylases of the endosperm, detected during germination of the seeds, were found to originate simultaneously with the physiological event. During the germination period, α-amylase also appeared in maize scutellum and in wheat and oat roots. Oat and wheat endosperms contain other amylolytic enzymes, in addition to α-amylase, during germination. Moreover, the amylolytic activities in the seeds, coleoptiles, and first leaves of maize, oats, and wheat were shown to be due to enzymes other than α-amylases from the endosperms of germinated seeds.

Isoenzymes of the amylases of seedlings of oats (*Avena* sp.) and rice (*Oryza* sp.) at germination and other stages of growth have been examined by disc electrophoresis.[195]

The levels of α- and β-amylase activities of excised cotyledons of the pea plant (*Pisum sativum*) have been found to increase during

[193] D. E. Briggs and V. J. Clutterbuck, *Phytochemistry*, 1973, **12**, 1047.
[194] V. Alexandrescu and F. Mihailescu, *Rev. Roumaine Biochem.*, 1973, **10**, 89.
[195] S. V. Iliev, *Doklady Bolg. Akad. Nauk*, 1973, **26**, 117.

incubation.[196] This increase could be inhibited by abscisic acid, but inhibition is not the result of a general inhibition of metabolism. Experiments with endogenous abscisic acid have indicated that the formation of both α- and β-amylases in germinating peas is regulated by the acid.

One form of α-amylase and two forms of β-amylase have been extracted from the pulps of Pass-Crassane pears, and the enzymes were partially purified.[197] A satisfactory extraction of the enzymes could be achieved only by use of non-ionic detergents. The three enzymes were characterized after separation by gel filtration.

Rye (*Secale cereale*) seeds have been shown to contain five species of α-amylase, which are separable by electrophoresis.[198] Three of these forms (A-forms) behaved as typical α-amylases, whereas the other two (B-forms) differed in their physicochemical characteristics. The B-forms were partially inactivated at pH 3.3 and 70 °C, but were more resistant to H_4edta than the A-forms. On electrophoretic examination, the A-forms migrated at lower rates than the B-forms. The activities of the various enzymes on maltose and starch were investigated. Aleurone layers were shown to contain large amounts of the A-forms. The relative amounts of the A- and B-forms were found to depend on the temperature of germination, but the B-forms remained active for a longer period after germination than the A-forms. Barley (*Hordeum vulgare*) and wheat (*Triticum vulgare*) seeds have been found to contain α-amylases similar to the A- and B-forms of rye.

The amylase isoenzymes in extracts from the seeds of wheat (*T. aestivum*) at various times during germination and in extracts from the roots, coleoptiles, first leaves, and stems have been separated and identified by electrophoresis.[199] During the first day of germination, thirteen isoenzymes (*viz.* ten α-amylases and three β-amylases) were recognized, and all of these enzymes could be inhibited by mercury(II) ions. As many as sixteen isoenzymes (*viz.* seven α-amylases and nine β-amylases) were detected on the third day of germination. As germination proceeded further, a number of enzymes not inhibited by mercury(II) ions were formed, and, ultimately, only isoenzymes of α-amylase could be detected. As many as ten isoenzymes of amylase could be detected in the roots and coleoptiles. Whereas all the root amylases could be inhibited by mercury(II) ions, four of those from the coleoptile were not susceptible to this inhibitor. Fewer isoenzymes of amylase were established in the first leaves, but all were inhibited by mercury(II) ions. A number of isoenzymes of amylase occur in the stem, but they exhibited comparatively lower activities.

Aqueous extracts of the flour of hard red spring wheat (*T. aestivum*) have been shown to contain two inhibitors of α-amylase, which were

[196] H. Yomo and J. E. Varner, *Plant Physiol.*, 1973, **51**, 708.
[197] J. C. Pech, G. Bonneau, and J. Fallot, *Phytochemistry*, 1973, **12**, 299.
[198] S. Wagenaar and T. F. Lugtenborg, *Phytochemistry*, 1973, **12**, 1243.
[199] S. V. Iliev, *Doklady Akad. Nauk Belorussk. S.S.R.*, 1972, **25**, 1417.

separated by ion-exchange chromatography.[200] The major inhibitor (molecular weight 2×10^4) was homogeneous on disc gel electrophoresis and possessed a p*I* of 6.7, whereas similar examination of the minor inhibitor revealed the presence of two contaminants. The inhibitors were classified as typical wheat albumins. A third inhibitor of α-amylase was discovered when it was observed that an albumin that is found only in *T. aestivum* varieties of wheat could inhibit the α-amylase obtained from chicken pancreas. The three inhibitors are distinguishable by their electrophoretic characteristics.

The α-amylase activity of the coleoptiles of wheat (*T. durum*) and the effects of treating the wheat with kinetin and gibberellic acid have been investigated.[201]

Other studies on developing grains of wheat have revealed the presence of four distinguishable types of amylase activity.[202] As determined from the amylograph, a very high level of amylase activity was found to be associated with the earliest stages of development, and with the outer layers, of the grain. Two other activities, determined by saccharogenic breakdown of soluble starch, were distinguished by their relative thermal stabilities. During the development of wheat grain in three successive years, these activities gave patterns characteristic of their cultivars. No thermostable activity remained at ripeness, but some thermolabile activity persisted beyond ripeness. Activities similar to those of wheat were found in barley and, to a lesser extent, in oats.

The production of a new, heat-stable α-amylase of bacterial origin, which is active and stable in starch solutions at temperatures above 100 °C, has been developed.[203] New liquefaction methods can be envisaged using this enzyme, since it is active over broader temperature and pH ranges, and is less dependent on the presence of copper(II) ions, than other α-amylases. Wheat and maize starches were liquefied in pilot-plant experiments using a reaction time of 2 h at 105 °C. Because of the higher temperatures at which the enzyme can be added (*ca.* 100 °C), the risk of retrogradation is greatly reduced compared with the normal liquefying techniques using commercial α-amylases.

The literature on the primary structure of the carbohydrate moiety of Taka-amylase A (one of the α-amylases of *Aspergillus oryzae*) has been reviewed.[204] Glycopeptides have been obtained from reduced and carboxymethylated Taka-amylase A by digestion with trypsin and chymotrypsin A.[205] The amino-acid sequences of the glycopeptides were determined, and the sequence (2) of a glycopeptide obtained by digestion with trypsin

[200] R. M. Saunders and J. A. Lang, *Phytochemistry*, 1973, **12**, 1237.
[201] A. Nougarède, P. A. Simonin, and P. E. Pilet, *Compt. rend.*, 1973, **276**, *D*, 3299.
[202] P. Meredith and L. D. Jenkins, *Cereal Chem.*, 1973, **50**, 243.
[203] G. B. Madsen, B. E. Norman, and S. Slott, *Stärke*, 1973, **25**, 304.
[204] R. Montgomery in 'Carbohydrates', ed. G. O. Aspinall, MTP International Review of Science, Organic Chemistry Series One, Butterworths, London, 1973, Vol. 7, p. 213.
[205] S. Isemura and T. Ikenaka, *J. Biochem.* (*Japan*), 1973, **74**, 1.

supports the suggestion that the asparaginyl residue linked to the 2-acetamido-2-deoxy-D-glucosyl residue of carbohydrate moiety is followed in the sequence by another residue and then by either threonine or serine residues.

Asn-Glu-Trp-Tyr-Asp-Trp-Val-Gly-Ser-Leu-Val-
Ser-Asn-Tyr-Ser-Ile-Asp-Gly-Leu-Arg
|
carbohydrate
(2)

The actions of 4-phenylazobenzoyl-Taka-amylase A, formed by reaction of the enzyme preparation with 4-phenylazobenzoyl chloride, on phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside (phenyl α-maltoside) and malto-oligosaccharides have been compared with those of the native enzyme.[206] With the α-maltoside, the modified enzyme liberated more phenol and less phenyl α-D-glucopyranoside than the natural enzyme, whereas the actions of the two enzymes on maltopentaitol were similar. It was concluded that the 4-phenylazobenzoyl group is positioned in the enzyme molecule very close to the catalytic site and that its interaction with the phenyl residue of the substrate of smaller molecular weight results in different actions for the two forms of the enzyme. Further studies of 4-phenylazobenzoyl-Taka-amylase A showed that, whereas the overall α-amylase activity was less than that of the natural enzyme, the derivatization did produce an absolute increase in the activity towards phenyl α-maltoside.[207] When the enzyme derivative was stored in the dark at 4 °C and pH 6, a proportion of the 4-phenylazobenzoyl groups were slowly liberated, with restoration of the action pattern to that of the natural form.

Using the hydrolysis of linear oligosaccharides catalysed by Taka-amylase A as a model, an attempt has been made to predict theoretically and quantitatively the changes in action pattern that accompany modification of one of the subsite affinities of a polymer-degrading enzyme.[14] The Taka α-amylase was chosen since all the subsite affinities had been determined previously. Calculations showed that a variety of action patterns could result from chemical modification at one of the subsites and that, in certain cases, enzymic activity towards smaller substrates increases, whereas that towards larger substrates decreases.

The effect of the level of active enzyme in the culture medium on the localization of, and the regulation mechanism for, the biosynthesis of α-amylase by *A. oryzae* has been studied.[208]

Amylase activity was among six glycosidase and glycanase activities detected in the purification of xylanases from a *Basidomycetes* sp. by ion-exchange chromatography and gel filtration.[139]

[206] K. Omichi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1972, **72**, 665.
[207] K. Omichi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1973, **73**, 491.
[208] V. V. Iurkevich and G. T. Kozyreva, *Doklady Akad. Nauk S.S.S.R.*, 1973, **209**, 1461.

The production of 'alkaline' α-amylases by alkaliphilic bacteria (a number of strains of *Bacillus* sp.) has been investigated.[209] Four α-amylase activities with different pH–activity profiles were detected in the culture fluids; the four forms (A—D) exhibited maxima in the pH–activity curves at pH values of 10, 4 and 10, 4 and 9, and 4 (with a shoulder at 10), respectively. Type-A was completely adsorbed by DEAE-cellulose, whereas the other types could be mixtures, although type-C exhibited the same pH–activity profile on purification by fifty-fold. Type-A α-amylase was thermolabile, whereas the others were fairly stable to heat. All four forms were stabilized by calcium ions, and the effect of variations in pH on the stabilities of the α-amylases was investigated.

The extracellular amylase of *Bacillus caldolyticus* has been purified from other macromolecules present by the use of a Diaflo ultrafiltration system.[210] Most of the total amylase activity was contained in a fraction in the molecular-weight range $1—3 \times 10^4$. The native enzyme appears to consist of a number of subunits, which, after extensive flushing, accumulated in the fraction with this molecular-weight range. During purification of fractions precipitated with ammonium sulphate, the amylase activity was found to decrease, but it could be restored by the addition of Ca^{2+} ions. Variation of the amounts of calcium ion necessary for full restoration of activity with temperatuture and the effect of calcium ions on the thermostability of the amylase were investigated.

The production of α-amylase by a strain of *Bacillus licheniformis* has been studied under conditions of batch and continuous culture.[211] Synthesis of the enzyme was repressed by D-glucose and other metabolizable sugars of low molecular weight. Consequently, production of α-amylase in a medium containing 'liquefied' starch began only after the carbohydrates of low molecular weight had been dissimilated. Thereafter, dextrins in the medium were degraded by the α-amylase produced by the bacteria to yield further quantities of metabolizable carbohydrates, which never accumulated in concentrations sufficient to repress the production of the enzyme. No evidence was obtained to infer that an inducer is necessary for production of the enzyme, and it was concluded that the principal factors influencing its production are the rate of growth and the regression of catabolites.

A strain of *Bacillus licheniformis* isolated from soil was found to produce extracellular α-amylase(s) having unusual characteristics.[212] The enzyme was purified by adsorption on to starch, treatment with DEAE-cellulose, and ion-exchange chromatography. Four active protein bands were detected by polyacrylamide disc gel electrophoresis in the final preparation, although the enzyme behaved as a single component during ultra-

[209] M. Yamamoto, Y. Tanaka, and K. Horikoshi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1819.
[210] J. A. Grootegoed, A. M. Lauwers, and W. Heinen, *Arkiv Mikrobiol.*, 1973, **90**, 223.
[211] J. L. Meers, *J. Microb. Serol.*, 1972, **38**, 585.
[212] N. Saito, *Arch. Biochem. Biophys.*, 1973, **155**, 290.

centrifugation and chromatography on either carboxymethylcellulose or Sephadex. The enzyme exhibited a very broad pH–activity profile and possessed substantial activity in the alkaline range. The optimum temperature for the enzyme was found to be 76 °C at pH 9, but it was stable in the pH range 6—11 at 25 °C and at pH 8 up to 60 °C. Gel filtration indicated that the α-amylase possesses a molecular weight of 2.25×10^4. The action pattern of the α-amylase on amylose and amylopectin is unique in that the predominant product during all stages of hydrolysis is maltopentaose. The immunological and electrophoretic properties of a purified preparation of α-amylase from *Bacillus licheniformis* have been compared with those of a commercial, crystalline preparation of α-amylase from *B. subtilis*.[213] The former enzyme reacted completely with the homologous rabbit antiserum to give a single precipitin band and it moved towards the cathode on immunoelectrophoresis on agarose at pH 9.6. By contrast, the α-amylase from *B. subtilis* cross-reacted only weakly with antisera to the *B. licheniformis* enzyme and it migrated to the anode on immunoelectrophoresis at pH 8.6, indicating that the two α-amylases are not identical. In addition, an immunological neutralization test showed that the enzyme from *B. licheniformis* is much more susceptible to inhibition by the antiserum than the commercial preparation. Each of the four distinct species of *B. licheniformis* α-amylase extracted from the polyacrylamide gel was shown to be antigenically similar to one another and to the parent enzyme.

α-Amylase from *Bacillus stearothermophilus* grown at 55 °C has been separated into two isoenzymes by ion-exchange chromatography; the major component exhibited greater stability than the minor one.[214] The properties of the two enzymes were compared with those of an α-amylase from the same bacterium grown at 43 °C. The latter enzyme proved to be similar to the major α-amylase produced at 55 °C with respect to such properties as molecular weight, amino-acid composition, electrophoretic properties, thermostability, pH optimum (pH 5), and c.d. spectrum. The minor species produced at 55 °C exhibited maximum activity at pH 5—6 and a higher activity than the other enzymes at alkaline pH values; it is considered not to be a proteolytic product derived from the major component during culturing and purification. The molecular weights of the three α-amylases from *B. stearothermophilus* were determined by gel filtration and sedimentation experiments.[215] The α-amylase produced at 43 C° contained both monomeric and dimeric forms; to eliminate the effects arising from dimerization, the enzymes were reduced and carboxymethylated, and the molecular weights were measured in solutions of guanidine hydrochloride. The molecular weights of the α-amylases produced at 43 and 55 °C were then found to be 4.4×10^4, 4.6×10^4 (major), and 4.3×10^4 (minor)

[213] N. Saito, *Arch. Biochem. Biophys.*, 1973, **159**, 409.
[214] K. Yutani, I. Sasaki, and K. Ogasahara, *J. Biochem.* (*Japan*), 1973, **74**, 573.
[215] K. Yutani, *J. Biochem.* (*Japan*), 1973, **74**, 581.

(by sedimentation), and 4.1×10^4, 4.4×10^4, and 4.1×10^4 (by gel filtration on Sepharose), respectively.

The effects of phosphate ion on sporulation and production of α-amylase by *Bacillus subtilis* have been investigated.[216] Growth of the organism and production of α-amylase were both promoted by the same concentration of phosphate ion, and curves relating the response of the organism to the concentration of phosphate ion were obtained. The intracellular α-amylase activity was less than 1% of the extracellular activity, and there seemed to be a reciprocal correlation between the concentration of phosphate ion and the amount of intracellular α-amylase. The amount of the α-amylase, as a catabolite-repressible enzyme, produced by *B. subtilis* growing in a chemostat was found to depend upon the growth-limiting substrate.[217] Limiting growth with D-glucose was advantageous for the biosynthesis of α-amylase. Under the conditions used, continuous cultures proved to be unsuitable for large-scale production of the enzyme, since spontaneous mutations to less-productive strains occurred in the long term. Mutants capable of increasing simultaneously the production of α-amylase and protease have been isolated from a transformable strain of *B. subtilis*.[218] The site of mutation was investigated, and a synergistic effect of this mutation and a regulatory gene on the production of extracellular α-amylase was observed.

The subunit structure of α-amylase from *Bacillus subtilis* has been studied by gel filtration and gel electrophoresis (in the presence of sodium dodecyl sulphate).[219] The crystalline form of the enzyme was found to be a tetramer (molecular weight 9.6×10^4) containing Zn^{2+} ions. Incubation of this species either at pH 5.5 or with H_4edta produced a zinc-free dimer (molecular weight 4.8×10^4), whereas incubation with sodium chloride at pH 7 produced a trimer (molecular weight 7.2×10^4) containing Zn^{2+} ions, and incubation at pH 8.5 produced a dimer (molecular weight 4.8×10^4) containing Zn^{2+} ions and a zinc-free monomer (molecular weight 2.4×10^4). Incubation of the tetramer at pH 7 had no effect. After standing, the Zn^{2+}-dimer formed insoluble aggregates that could be dissolved by treatment with H_4edta. The aggregates possessed molecular weights in the range $1.25—4.0 \times 10^5$. The Zn^{2+}-trimer also aggregated to form a single species (molecular weight 1.44×10^5). All molecular forms of the enzyme are active, although they differ widely in their specific activities.

α-Amylase (from *Bacillus subtilis*) from which the calcium content had been removed could be reactivated under controlled conditions by lutetium(III) ions and a variety of other ions from the lanthanide series.[220] Reactivation was extremely sensitive to the concentration of lanthanide ions and to the nature of the buffer used. Thus, at pH 6.9 in Hepes

[216] P. M. Markkanen and T. Enari, *Acta Chem. Scand.*, 1972, **26**, 3543.
[217] F. G. Heineken and R. J. O'Connor, *J. Gen. Microbiol.*, 1973, **73**, 35.
[218] Y. Yoneda, K. Yamane, and B. Maruo, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 765.
[219] J. F. Robyt and R. J. Ackerman, *Arch. Biochem. Biophys.*, 1973, **155**, 445.
[220] D. W. Darnall and E. R. Birnbaum, *Biochemistry*, 1973, **12**, 3489.

(*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) buffer solutions, concentrations of lutetium ion above 10^{-4} mol l^{-1} inhibited the enzyme, whereas, in maleate buffer at pH 6.9, the apo-α-amylase was activated by concentrations of lutetium ion up to 10^{-3} mol l^{-1}. An investigation of the action of α-amylase from *B. subtilis* on 6-deoxy-6-iodoamylose showed that the enzyme did not liberate monomeric 6-deoxy-6-iodo-D-glucose.[221] Chromatography, enzymic analyses, g.l.c., and mass spectrometry of volatile derivatives of the products indicated that the smallest oligomer liberated by the α-amylase is 6^2-deoxy-6^2-iodomaltotriose. The position of the iodo-group in this product suggested that it has the same effect as a branch point in amylopectin, where 6^2-α-maltosylmaltotriose is the smallest branched molecule produced.

The effect on the thermal stability of acylation of the α-amylase from *Bacillus subtilis* with 4-nitrophenylacetate has been studied.[222] The thermostability of the enzyme was increased by acylation at temperatures above 70 °C, whereas it was decreased at temperatures below 67 °C. A compensation effect was also observed for heat inactivation of the acylated α-amylase and, in this case, the temperature of compensation is 68 °C. This effect seems to be due to a change of conformation caused by acylation. The significance of the compensation effect in studies of the denaturation and the stability of proteins was discussed.

Immobilized derivatives of α-amylases have been produced by covalent attachment of the enzymes to titanium complexes of poly-(4- and 5-acrylamidosalicylic acids)[223] and by photopolymerization of aqueous solutions containing the enzyme, acrylamide, a cross-linking monomer (allyl glycidyl ether), an enzyme-linking monomer (glycidyl acrylate), and an oxygen scavenger; in the latter case, the enzyme reacted with the gel matrix evolved.[224]

β-Amylases

Treatment of Amylopectin Azure (a dyed amylopectin available commercially) with β-amylase to give the β-limit dextrin afforded a substrate that is entirely specific for α-amylase activity.[179] The β-limit dextrin derivative has been used as the basis of a simple method for distinguishing between α- and β-amylases. The presence of β-amylase in the α-amylase did not significantly affect the release of dyed carbohydrates from the substrate.

Determination of the anomeric configuration of the products resulting from the action of a carbohydrase on its substrate has been based on the observation that the sign of the accumulation rate of the P_1-anomer in the process

$$\text{substrate} \xrightarrow{\text{enzyme}} \text{anomer } P_1 \rightleftharpoons \text{anomer } P_2$$

[221] C. E. Weill and J. Guerrera, *Carbohydrate Res.*, 1973, **27**, 451.
[222] I. Urabe, H. Nanjo, and H. Okada, *Biochim. Biophys. Acta*, 1973, **302**, 73.
[223] J. F. Kennedy and J. Epton, *Carbohydrate Res.*, 1973, **27**, 11.
[224] H. M. Walton and J. E. Eastman, *Biotechnol. and Bioeng.*, 1973, **15**, 951.

can change under certain conditions, whereas that of the P_2-anomer remains positive.[21] A kinetic study using ^1H n.m.r. spectroscopy of the stereochemically known hydrolysis of amylopectin by β-amylase provided evidence for the validity of conclusions based on accumulation rates. It was also pointed out that the direct spectroscopic procedure has a number of limitations that are overcome by the kinetic approach.

The levels of α- and β-amylase activities of excised cotyledons of the pea (*Pisum sativum*) were found to increase during incubation.[196] These increases are inhibited by abscisic acid, but inhibition is not the result of a general inhibition of metabolism. Experiments with endogenous abscisic acid suggested that the formation of α- and β-amylases in germinating peas is regulated by the acid.

Two β-amylases and one α-amylase have been extracted from pulps of Pass-Crassane pears, and the enzymes were partially purified.[197] The greatest amounts of the activities were extracted with non-ionic detergents, and such systems were essential for solubilization of one of the β-amylases. The three enzymes were separated by gel filtration, and the β-amylases were found to possess similar kinetic properties, and interconversion of one form into the other is possible. It was suggested that the insoluble form arises from association of the other form with compounds present in pear fruits.

Avoidance of side-effects due to an α-glucosidase impurity in preparations of α-amylase from sweet potato by the use of erythritol or 2-amino-2-hydroxymethylpropane-1,3-diol is reported to have inherent disadvantages.[185] Ion-exchange chromatography of the β-amylase preparation on DEAE-Sephadex is recommended, since this completely separates the two activities. The recovered β-amylase was shown to be inactive towards maltose, despite earlier reports to the contrary. The action of β-amylase from sweet potato on glycogen has also been investigated.[188]

Studies of the effect of sodium dodecyl sulphate on the structure of β-amylase from sweet potato have shown that the decrease in activity caused by the reagent is followed by an increase in the number of titratible SH groups.[225] The protein species observed on gel filtration and on polyacrylamide gel electrophoresis of the denatured enzyme are considered to correspond to monomeric and polymeric forms, respectively.

Using β-amylase from sweet potato, the number of bonds cleaved for each effective enzyme–substrate encounter has been determined for the β-amylolysis of amyloses having various degrees of polymerization and labelled enzymically from D-[^{14}C]glucose 1-phosphate.[226] The type of degradation was found to shift in the direction of a 'single-chain' mechanism with increasing degree of polymerization of the substrate. By taking into account the 'inactive, non-reactive' collisions leading to 'self-inhibition',

[225] Y. Takeda, S. Hizukuri, and J. Shmada, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 367.
[226] E. László, J. Holló, and B. Bánky, *Carbohydrate Res.*, 1972, **25**, 355.

the number of bonds cleaved per 'reactive' and 'non-reactive' enzyme–substrate encounter was established. From these data and from the inhibition constant of inner D-glucose residues of the amylose chain, in conjunction with the Michaelis constant extrapolated to a DP of 4, the actual rate constants for formation and dissociation of 'reactive' and 'inactive' enzyme–substrate complexes were determined. The rate constants for formation of the 'reactive' complexes were found to decrease slowly with increasing extents of 'self-inhibition', whereas those for dissociation of the complexes decrease to a greater extent.

The inhibition of the β-amylase of sweet potato by cycloamyloses has been studied.[227] Cyclohexa-amylose was found to be a considerably more powerful inhibitor of the enzyme than cyclohepta-amylose. Inhibition of β-amylase by these cycloamyloses was substantially reduced in the presence of DMSO, and this effect was related to a change in conformation that takes place with cycloamyloses in DMSO. C.d. measurements indicated that no change in the conformation of β-amylase occurred on interaction with cyclohexa-amylose. It was concluded from these studies that an induced-fit mechanism is not involved in the action of β-amylase. Evidence supporting a distortion of the sugar ring during catalysis by β-amylase, such as takes place in lysozyme-catalysed hydrolyses, was presented.

Electrophoretic analysis of the amylases of ungerminated seeds of rye (*Secale cereale*) has provided evidence for the presence of two β-amylases; the two enzymes, however, could not be detected in germinated seeds.[198] The existence of β-amylases in the seeds of germinated barley (*Hordeum vulgare*) and wheat (*Triticum vulgare*) was also reported on the basis of electrophoresis. The isoenzymes of amylase present in extracts from seeds of wheat (*Triticum aestivum*) at various stages of germination and in extracts of roots, coleoptiles, first leaves, and stems have been separated and identified by electrophoresis.[199] Of the thirteen isoenzymes identified on the first day of germination, three are iso-enzymes of β-amylase which, like the accompanying isoenzymes of α-amylase, were inhibited by mercury(II) ions. On the third day of germination, nine isoenzymes of β-amylase were recognized, but as germination proceeded only isoenzymes of α-amylase remained.

β-Amylase has been immobilized by covalent fixation to amino-derivatives of Sepharose cross-linked with epichlorohydrin, mediated by cyclohexyl isocyanide and acetaldehyde.[228] Columns of the immobilized enzyme were used for the continuous degradation of starch.

Amylo-1,6-glucosidases (Dextrin-1,6-glucosidases)

Studies of the subunit structures of rabbit-muscle and yeast glucosidase-transferases have been conducted by gel electrophoresis of the amylo-1,6-glucosidase-4-α-glucanotransferase systems in the presence of sodium

[227] J. J. Marshall, *European J. Biochem.*, 1973, **33**, 494.
[228] P. Vretblad and R. Axén, *Biotechnol. and Bioeng.*, 1973, **15**, 783.

dodecyl sulphate.[229] The rabbit-muscle enzyme gave components having molecular weights of 1.18×10^5 and 9.4×10^4, whereas those from yeast have molecular weights of 1.18×10^5, 8.3×10^4, and 6.6×10^4. On carboxymethylation prior to electrophoresis (to ensure complete dissociation), the corresponding values for the enzyme from rabbit muscle were 1.20×10^5 and 9.2×10^4, and for the enzyme from yeast 1.22×10^5, 8.5×10^4, and 7.0×10^4. Similar results were obtained on electrophoresis of the enzymes in the presence of urea. The substrate specificities of the glucosidase-transferase debranching enzymes from rabbit muscle and yeast have been examined, using polysaccharides of defined outer chain-lengths as substrates.[230] The results are consistent with specificities previously ascribed to the 4-α-glucanotransferase portion of the debranching enzyme on the basis of studies using malto-oligosaccharides as substrates. The specificities of the two enzymes were also examined in the reversion system, wherein both displayed an inverse specificity to that observed in hydrolytic reactions. This suggested that the reversion reaction reflects the specificity of the glucosidase portion of the debranching system. The major differences between the yeast and rabbit-muscle systems were found to lie in the specificity of the transferase and in the ability of the yeast system to debranch glycogen and amylopectin.

Carrageenases

An enzyme complex that hydrolyses the carrageenan extractable from the red alga *Chondrus crispus* with aqueous potassium chloride solution has been isolated from cell-free media of cultures of *Pseudomonas carrageenovora* grown on this polysaccharide.[231] The hydrolases of the complex could be classified. Fraction I caused a rapid decrease in the specific viscosity of solutions of the polysaccharide with only the minimal release of reducing sugars, and could be distinguished from fraction II by gel filtration and gel electrophoresis on agarose. Fraction IIa released reducing sugars from the polysaccharide at pH 6.2 (optimum), but not at pH 7.5, whereas fraction IIb exhibited activity at both pHs. The behaviours of solutions of the enzymes on freezing, dialysis, heating, and treatment with mercury(II) ions and H_4edta were examined. Incubation of fractions IIa and IIb at pH 6.2 with carrageenans yielded sulphated oligosaccharides containing no 3,6-anhydro-D-galactose residues. Examination of the substrate specificity for a range of carrageenans showed that λ- and μ-carrageenans are degraded by the enzyme, whereas κ-carrageenans are not.

Cellulases

Fractionation of the cellulase activity of snail (*Helix pomatia*) juice on DEAE-cellulose afforded the enzyme in a highly purified form, and evidence

[229] E. Y. C. Lee and J. H. Carter, *F.E.B.S. Letters*, 1973, **32**, 78.
[230] E. Y. C. Lee and J. H. Carter, *Arch. Biochem. Biophys.*, 1973, **154**, 636.
[231] K. H. Johnston and E. L. McCandless, *Canad. J. Microbiol.*, 1973, **19**, 779.

that DEAE-cellulose functioned as an affinity-chromatography matrix was presented.[232] *endo*-Cellulase has been separated from the other glucanases, including laminarinases, present in snail juice by molecular-sieve chromatography, and it was then further purified by affinity chromatography on DEAE-cellulose and by gel filtration.[233] The behaviour of the enzyme on DEAE-cellulose differed from that of a microbial cellulase studied previously, although the molecular weights (1.8×10^4) of the two enzymes appeared to be identical.

The presence of cellulase activity in Cephalopoda has been reported.[234]

Changes in the levels of various glucanases, including *endo*-β-1,4-glucanase (cellulase) activities, in the second internode of the stems of developing oat plants have been examined.[134] Concurrent changes in non-cellulosic β-glucans contained in the corresponding hemicelluloses were also studied. Possible relations between the observed changes and the growth and development of the plant tissues were discussed.

The cellulase activity of the separation-zone tissues of orange trees (*Citrus sinensis*) one day after treatment of the trees with cycloheximide has been investigated.[235] *exo*-1,4-β-Glucosidase (*exo*-cellulase) was detected only in the separation zones of treated fruit, whereas *endo*-cellulase activity was present in zones from both treated and control fruits, but was three times as great in treated fruit. In all cases, the cellulase activity was restricted to the separation-zone tissues, and the effect of cycloheximide is apparently mediated by ethylene produced by wound tissues.

Molecular-sieve chromatography of an extract from ungerminated seeds of rye has indicated the presence of enzymes that hydrolyse β-glucans and related oligosaccharides, including an *endo*-cellulase that hydrolyses carboxymethylcellulose.[137]

Cellulolytic enzymes have been studied in extracts from the locular contents of ripening fruits of the tomato plant (*Lycopersicum esculentum*).[138] When acting on carboxymethylcellulose, the enzyme was capable of decreasing the viscosity of the solution and of generating reducing groups, oligosaccharides, and D-glucose. Cello-biose, -triose, -tetraose, and -pentaose also served as substrates, with the formation of D-glucose. These properties are considered to be due to the presence in the fruit extracts of an *endo*-cellulase and a β-glucosidase; no evidence was found for the presence of an *exo*-1,4-β-glucosidase. The kinetics of the system were investigated, and cellobiose was demonstrated to be a weak inhibitor of the cellulase.

A cellulase preparation exhibiting maximum activity at an unusually low pH (2.3—2.5) has been obtained from a commercial cellulase produced

[232] J. J. Marshall, *Analyt. Biochem.*, 1973, **53**, 191.
[233] J. J. Marshall, *Comp. Biochem. Physiol.*, 1973, **44B**, 981.
[234] M. Furia, L. Gianfreda, and V. Scardi, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 1127.
[235] G. K. Rasmussen, *Plant Physiol.*, 1973, **51**, 626.

from culture filtrates of *Aspergillus niger*.[236] Purification of the enzyme by fractional precipitation with ammonium sulphate, gel filtration, and ion-exchange and adsorption chromatography gave a material that was homogeneous on ultracentrifugation and disc and ampholine electrophoreses. The 3.27 *S* enzyme was shown to be acidic, and to possess a p*I* of 3.3 and a molecular weight of 4.6×10^4. The u.v. and o.r.d. spectroscopic properties of the enzymes were investigated. Further studies of the cellulase from *A. niger* showed that it is stable within the pH range 4.0—10.0 and that it exhibits its highest activity towards glycolcellulose at pH 2.5.[237] The optimum temperature for enzymic activity was 50 °C, although the enzyme was inactivated by heating above 40 °C. Insoluble cellulose was not readily attacked by the enzyme, the activity of which was inhibited by magnesium(II) and manganese(II) ions and, to a slight extent, by cobalt(II) ions. The enzyme was found to contain arabinose (10), 2-amino-2-deoxyglucose (12), and amino-acids (378 moles per mole), but no *N*-terminus could be detected.

Eight alkyl oligo-(1 → 4)-β-D-glucopyranosides having terminal epoxide functions in the aglycone have been tested as irreversible inhibitors directed towards the active sites of cellulases from *Aspergillus niger*, *A. wentii*, and an *Oxyporus* species.[238] The oligosaccharide glycosides showed very little reactivity, but increasing deactivation was observed with increasing numbers of D-glucose residues. The aglycone showed maximum reactivity for a chain-length of five carbon atoms. These data and partial protection of the enzyme by cellobiose indicated that deactivation is due to attachment of the inhibitor to the active site. Variation of the rates of deactivation with pH was very similar to that of the cellulase activity, and, in each case, a carboxy-group seems to be involved as an essential acid catalyst. Another enzyme detected in the preparation from the *Oxyporus* species was recognized as a cellobiosidase, which liberated 4-nitrophenol from 4-nitrophenyl β-cellobioside.

Cellulase activity has been detected in a Basidiomycete species during fractionation of polysaccharidases of the micro-organism by ion-exchange chromatography.[139] Polysaccharidase fractions obtained from species of Basidiomycetes, *Fusarium*, *Trametes*, and *Trichoderma* were found to be active against carboxymethylcellulose, curdlan, and luteose, carboxymethylcellulose and luteose, carboxymethylcellulose, curdlan, and luteose, and carboxymethylcellulose, respectively.[239] An enzyme obtained from the culture medium of a *Flavobacterium* species grown on 'succinoglucan' (a polysaccharide containing D-galactose, D-glucose, and succinic acid)

[236] R. Ikeda, T. Yamamoto, and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1153.
[237] R. Ikeda, T. Yamamoto, and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1169.
[238] G. Legler and E. Bause, *Carbohydrate Res.*, 1973, **28**, 45.
[239] T. Harada, K. Moori, and A. Amemura, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2611.

from *Alcaligenes faecalis* as the source of carbon was found to be active against the succinoglucan, as well as a polysaccharide containing β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-linked D-glucose residues, and, to a slight extent, laminarin. Since growth of the organism on luteose produced an enzyme that was inactive against succinoglucan, whereas growth of the organism on D-glucose produced no polysaccharidase, it was concluded that the organism produces different substrate-specific β-glucanases according to the source of carbon used for growth.

Fractionation on microgranular carboxymethylcellulose has been reported as a new procedure for the purification of *endo*-cellulase from a *Cytophaga* species.[240] That interaction of the enzyme with the chromatographic matrix is solely of the affinity type was evidenced by the fact that little or no binding of the enzyme took place on carboxymethyl-Sephadex. The enzyme appears to be acidic, and it was found that it could also be purified by chromatography on DEAE-cellulose and -Sephadex. However, the highest specific activity of the purified enzyme was obtained using carboxymethylcellulose, reflecting a lower degree of interaction of the enzyme with a cation-exchanger than with an anion-exchanger.

Cellulolytic micro-organisms (eighteen bacterial and twelve fungal) isolated from samples of cotton, which had deteriorated during storage in Indian cotton mills, have been screened for the production of cellulase on various media.[241] *Penicillium funiculosum* was found to be rich in the C_1 component of cellulase, and the cellulase activity is comparable to that of *Trichoderma viride*.

Rhynchosporium secalis grown in liquid culture media containing single soluble or insoluble cellulosic sources of carbon liberated a complex of cellulase enzymes into the media.[242] The complex was separated by gel filtration and ion-exchange chromatography into three fractions of high molecular weight and three fractions of low molecular weight. Each of the individual fractions liberated D-glucose when applied to insoluble cellulose, but the individual activities were always lower than that obtained by the combination of certain fractions. Such a combination resulted in a large synergistic increase in activity against the substrate.

Factors affecting the digestion of pebble-milled cellulose by enzymes have been studied by using several strains (including a mutant) of *Ruminococcus albus*, most of the cellulase activities of which are extracellular.[243] As much as 65% of the cellulose was digested by the cell-free enzymes, provided that the quantity of cellulose was small. Cellobiose and, to a lesser extent, D-glucose inhibited the digestions. Carboxymethylcellulose was digested more rapidly than pebble-milled cellulose, but only to approximately the same final extent (as judged by copper-reduction values). Cell walls of alfalfa were also digested by the enzyme. The

[240] J. J. Marshall, *J. Chromatog.*, 1973, **76**, 257.
[241] A. J. Desai and S. M. Betrabet, *Indian J. Biochem. Biophys.*, 1972, **9**, 212.
[242] P. O. Olutiola and P. G. A. Ayres, *Trans. Brit. Mycol. Soc.*, 1973, **60**, 273.
[243] W. R. Smith, I. Yu, and R. E. Hungate, *J. Bacteriol.*, 1973, **114**, 729.

cellulase exhibited maximum activity over the pH range 6.0—6.8, and digested cellulose most rapidly at 45 °C; part of the cellulolytic activity was irreversibly destroyed on exposure to oxygen. Much of the enzymic activity was adsorbed on to the cellulosic substrate, and the characteristics of the adsorption–desorption processes, together with partial inhibition brought about by oxygen, indicated that multiple enzymes are involved.

The production of cellulolytic enzymes by virulent and avirulent isolates of *Sclerotium bataticola* during the development of disease in sunflowers (*Helianthus annuus*) has been investigated.[244] The cellulase activity in the plant extracts increased with time after inoculation and, whereas the cellulase is not important in the penetration of *S. bataticola* into sunflowers, it is involved in further development of the pathogen. The ability to produce cellulase, *exo*-polygalacturonase, and polygalacturonase in sunflower plants is related to the virulence of the isolates.

The growth characteristics of *Thermomonospora fusca*, a thermophilic cellulolytic actinomycete, on pulping fines (a cellulosic waste of the paper industry) have been reported.[245] Using the organism, a fermentation process was developed that substantially degraded this waste, and the residual product of growth contained approximately 30% microbial protein. Feeding experiments showed that the protein is of good nutritional quality and that it contained no strongly toxic materials. The possible recovery of extracellular cellulolytic enzymes from the spent broth and the possible use of the fermentation process as a waste-disposal system were discussed.

A cellulase complex of *Trichoderma koningii* has been separated into four apparently pure components: *viz.* a β-glucosidase, a C_1 component, and two new components [a cellulase active against carboxymethylcellulose (C_a) and a component designated C_2].[146] All four components were necessary for the efficient solubilization of natural cellulose, and the two new components constituted the composite short-fibre-forming activity of the complex.

A procedure for determining the digestibility of cellulose, wood, and modified forms thereof has utilized a cellulolytic enzyme preparation from *Trichoderma viride*.[246] Fine grinding was the most effective way of increasing the digestibility of the substrate, and the overall procedure was used as a screening test for evaluating the effects of various physical and chemical treatments aimed at improving the accessibility of the cellulosic chains to ruminants.

Cellobiosidases

An enzyme preparation from an *Oxyporus* species was found to contain cellobiosidase activity that is able to hydrolyse 4-nitrophenyl β-cellobioside

[244] Y. H. Chan and W. E. Sackston, *Canad. J. Botany*, 1972, **50**, 2449.
[245] D. L. Crawford, E. McCoy, J. M. Harkin, and P. Jones, *Biotechnol. and Bioeng.*, 1973, **15**, 833.
[246] W. E. Moore, M. J. Effland, and M. A. Millett, *J. Agric. Food Chem.*, 1972, **20**, 1173.

to cellobiose and 4-nitrophenol.[238] This enzyme is considered to be distinct from cellulases present in the same preparation, since it was not significantly affected by inhibitors of the cellulases. The cellobiosidase is also considered to be distinct from β-glucosidase, since it was not affected by (1,3/2,4)-cyclohex-5-ene-1,2,3,4-tetraol epoxide, a compound known to be a specific inhibitor of β-glucosidase activity.

Chitinases

Chitin has been prepared in colloidal form as a substrate for chitinase.[247] The colloidal chitin was used to detect chitinolytic activities in secretions of a *Nepenthes* species and in an extract of *Drosera pedata*. In both cases, 2-acetamido-2-deoxy-D-glucose was identified among the products of enzymic action. It is uncertain whether the chitinolytic enzymes are formed by the plant itself or by a symbiotic micro-organism, but the ability of these plants to digest insects completely is considered to arise from a combination of chitinolytic activity and a number of proteases.

Affinity chromatography of saliva and extracts of taro and yam on an affinity column of chitin-coated cellulose yielded, in each case, a specifically adsorbed fraction that was very active against glycol-chitin.[248] Only in the case of the yam extract did the non-specific fraction also contain chitinolytic activity. However, in the cases of saliva and the taro extracts, the specifically adsorbed fractions possessed slight activity against bacterial substrates. The yam chitinase fraction gave a single peak on gel filtration.

The distribution of chitinolytic activity among eighty-two strains of Basidiomycete species has been determined, but only low activities were detected.[249]

Dextranases

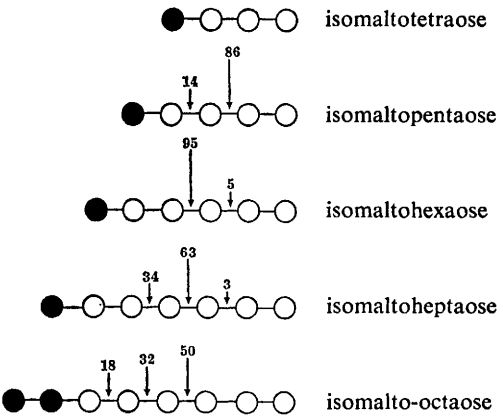
Use of dextranase to remove interference caused by dextran in the determination of serum proteins by the Biuret reaction has been advocated.[250]

A bacterium from soil has been shown to induce at least two extracellular dextranases (and also some intracellular dextranase activity) when grown on a medium containing dextran as the sole source of carbon.[251] Fractional precipitation with ammonium sulphate and gel filtration yielded a dextranase, which behaved as a single component on polyacrylamide electrophoresis at two pH values, and another fraction containing dextranase activity, which gave two protein bands on electrophoresis. Both enzymes yielded mixtures of oligosaccharides from dextran and are regarded as *endo*-enzymes. The effects of pH on the enzymic activities, the thermal stabilities of the enzymes, and their temperature optima (42 and 55 °C,

[247] S. Amagase, M. Mori, and S. Nakayama, *J. Biochem. (Japan)*, 1972, **72**, 765.
[248] T. Imoto and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1191.
[249] M. Kawi, *J. Agric. Chem. Soc. Japan*, 1973, **47**, 473.
[250] S. M. Nazar and H. Schmidt, *Z. klin. Chem. klin. Biochem.*, 1972, **10**, 548.
[251] G. N. Richards and M. Streamer, *Carbohydrate Res.*, 1972, **25**, 323.

respectively) were investigated. Both enzymes exhibited broad pH–activity profiles. An intracellular dextranase preparation obtained from the same soil bacterium degraded dextran principally to D-glucose, but was apparently unable to attack certain non-(1 → 6)-linked D-glucose residues.[252] The initial attack of the enzyme on dextran appeared to be *endo*, but attack on linear isomaltodextrins appeared to be *exo*, one D-glucose unit at a time being liberated from the non-reducing end of the substrate. The pH and temperature optima of the enzyme and its stability to heat were investigated.

A series of isomalto-oligosaccharides, containing terminal, non-reducing D-[^{14}C]glucopyranosyl units, have been used to determine the specificity of a bacterial dextranase that requires five or more consecutive α-(1 → 6)-D-glucosidic linkages in the substrate before its activity is manifested.[253] The proportion of attack at each glycosidic linkage of the substrate was calculated from the distribution of radioactivity among the products (see Scheme 6). The third linkage from the reducing end of isomaltohexaose



○ D-glucose residue
● D-[^{14}C]glucose residue
— α-(1 → 6)-D-glucosidic linkage
Arrows indicate the point and extent of hydrolysis (%).
Linkages that are not arrowed are resistant to dextranase.

Scheme 6

and substrates with longer chains (isomaltoheptaose and isomalto-octaose) was shown to be the most susceptible to attack by the enzyme. Isomaltopentaose was hydrolysed to isomaltose and isomaltotriose when a large excess of enzyme was used, but these oligosaccharides and isomaltotetraose

[252] N. W. H. Cheetham and G. N. Richards, *Carbohydrate Res.*, 1972, **25**, 333.
[253] G. J. Walker, *Carbohydrate Res.*, 1973, **30**, 1.

were not further degraded. It was concluded that the dextranase hydrolyses linkages penultimate to either end of the substrate chain only with difficulty and that terminal linkages are completely resistant to attack.

Screening of twenty-eight *Penicillium* cultures for dextranase activity showed that maximum activity was produced by *P. aculeatum* and a strain of *P. funiculosum*.[254] The fermentation kinetics for production of the enzyme by the latter organism were studied, and the extracellular dextranase produced exhibited maximum activity at pH 5.0—5.5 and 55 °C. The crude dextranase inhibited the formation of insoluble glucan by *Streptococcus mutans*. The dextranase from *P. funiculosum* has been purified by means of gel filtration and ion-exchange chromatography.[255] Isoelectric focusing of the purified dextranase yielded two active fractions (I and II, p*I* values 4.0 and 4.2, respectively), both of which were electrophoretically homogeneous. The two dextranases possess the same molecular weight (4.4×10^4, by gel filtration) and pH optimum (6.0). Both dextranases were stable over the pH range 5.0—7.5 at 37 °C for one hour, and were activated by cobalt(II), manganese(II), and copper(II) ions, but were inactivated by silver(I) and mercury(II) ions, *N*-bromosuccinimide, and iodine. Further studies showed that dextranase II specifically hydrolysed dextrans with α-(1 → 6)-linkages, but it did not exhibit activity towards other polysaccharides.

Active, water-insoluble derivatives of dextranase have been prepared by covalent attachment of the enzyme to cellulose carbonate, carboxymethylcellulose azide, and cellulose cyclic imidocarbonate.[256] The use of these various matrices for immobilization of the enzyme was compared.

Galactanases

Three galactanases, two in crystalline form, have been obtained from culture broths of a strain of *Bacillus subtilis*, and the specificities and properties of the enzymes were compared.[257] Although the molecular weights of the enzymes proved to be similar, the isoelectric points of the proteinaceous molecules were considerably different. The galactanases exhibited maximum activities in the pH range pH 6—7, were sensitive to metal chelates, and were stabilized by calcium(II) ions. All three galactanases attacked the arabinogalactan of soybean without liberation of arabinose, but were inactive against coffee-bean arabinogalactan. In the digestion of the soybean arabinogalactan, the galactanases liberated D-galactose, galactobiose, and galactotriose, whereas a galactanase previously isolated from another strain of *B. subtilis* produced only galactobiose.

A novel glycosidase, which specifically hydrolyses a polysaccharide from *Salmonella anatum* consisting of α-galactosylmannosylrhamnosyl repeating

[254] N. Kosaric, K. Yu, J. E. Zajic, and J. Rozanis, *Biotechnol. and Bioeng.*, 1973, **15**, 729.
[255] M. Sugiura, A. Ito, T. Ogiso, K. Kato, and H. Asano, *Biochim. Biophys. Acta*, 1973, **309**, 357.
[256] N. W. H. Cheetham and G. N. Richards, *Carbohydrate Res.*, 1973, **30**, 99.
[257] S. Emi and T. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1945.

units, has been found to be associated with the *Salmonella* particle and may be responsible for its specific adsorption to bacteria.[258] The enzyme was assayed by determination of the increase in reducing power of the substrate; it was inhibited by bivalent cations. A β-galactosylmannosyl-rhamnosyl-type polysaccharide did not act as a substrate for the enzyme.

endo-β-1,3-Glucanases (Oligo-1,3-glucosidases)

Changes in the levels of various β-glucanase activities, including *endo*-β-1,3-glucanase, in the second internode of the stems of developing oat plants have been examined.[184] Concurrent changes in non-cellulosic β-glucans contained in the corresponding total celluloses were also determined. Possible relationships between the observed changes and the growth and development of the plant tissues were discussed.

An *endo*-β-1,3-glucanase has been obtained from an extract of ungerminated seeds of rye by fractional precipitation and gel filtration.[137] The enzyme was active against laminarin and pachyman (no D-glucose being liberated from either), laminaritriose (laminaribiose and laminaritetraose being formed, together with small amounts of D-glucose and laminaripentaose), and laminaritetraose (D-glucose, laminaribiose, and laminaritriose being formed). The enzyme was inactive against lichenin, barley β-glucan, oat glucan, cellodextrin, cellobiose, and laminaribiose, and was inhibited by phenylmercuric nitrate. The role of the enzyme *in vivo* was discussed.

An enzyme capable of hydrolysing laminarin has been purified from homogenates of suspension-cultured tobacco (*Nicotiana tabacum*) cells by means of ion-exchange chromatography and gel filtration.[259] The purified enzyme was homogeneous on disc electrophoresis and appeared to be a basic protein. The laminarinase exhibited maximum activity at pH 5 and was stable at temperatures up to 40 °C. Identification of the products of its action on laminarin revealed an *endo* action-pattern, the substrate-being degraded to D-glucose, laminari-biose, -triose, -tetraose- and -pentaose.

Polysaccharidase fractions from an *Arthrobacter* species, Basidiomycetes, and a *Trametes* species were each found to be active towards curdlan [a β-(1 → 3)-glucan].[239] The fractions from the two latter species were also shown to hydrolyse carboxymethylcellulose and luteose.

A *Bacillus* species isolated from soil in alkaline media produced a β-1,3-glucanase in the culture medium.[260] After purification on ion-exchange, gel-filtration, and hydroxyapatite columns, the enzyme was found to possess a molecular weight of 4×10^4 and an $S_{20,w}$ value of 3.6 *S*. The enzyme (p*I* 3.5) was most active at pH 5.5—8.0, which is a much broader pH optimum than that of a similar enzyme from *B. circulans*. The enzyme was most stable at pH 7, and calcium(II) ions were not

[258] K. Takeda and H. Uetake, *Virology*, 1973, **52**, 148.
[259] K. Kato, A. Yamada, and M. Noguchi, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1269.
[260] K. Horikoshi and Y. Atsukawa, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1449.

effective in stabilizing the enzyme. Laminaritetraose was hydrolysed by the enzyme to products that included D-glucose and laminaritriose, but the trisaccharide was not attacked. Laminarin was hydrolysed randomly by the enzyme to yield D-glucose, laminaritriose, laminaribiose, and higher oligosaccharides. On the assumption that the enzyme contains a single activity, it was concluded to be an *endo*-β-1,3-glucanase.

A commercial enzyme preparation from a *Cytophaga* species yielded three distinct β-glucanase fractions on ion-exchange chromatography on DEAE-cellulose.[261] One of the fractions was not adsorbed on the column and was found to be active towards laminarin only, with the liberation of higher oligosaccharides. The principal oligosaccharides possessed DPs of seven and eight, and the enzyme (molecular weight 1.8×10^4, by gel filtration) appeared to be a form of *endo*-β-1,3-glucanase. One of the other enzyme fractions appeared to be a laminarinase.

Fractionation (by ion-exchange chromatography) of enzymes secreted by protoplasts and intact cells of *Saccharomyces cerevisiae* revealed the presence of three species capable of hydrolysing laminarin.[143] The first fraction, which was not retained by the column, appeared to consist of two enzymes exhibiting activity towards laminarin, 4-nitrophenyl β-D-glucopyranoside, and pustulan (β-1,6-glucan), and a low, but detectable, activity towards periodate-oxidized laminarin. The second fraction (pH optimum 5.5) possessed the substrate specificity of an *endo*-β-1,3-glucanase and the third fraction contained an unspecific *exo*-β-glucanase. The properties of these extracellular β-glucanases were compared with those occurring in protoplast lysates and in cell-free extracts of intact cells of the organism.

endo-β-1,6-Glucanases

Polysaccharidase fractions from species of Basidiomycetes, *Fusarium*, and *Trametes* have been shown to be active towards luteose [a β-(1 → 6)-D-glucan].[239] Fractions from Basidiomycetes and *Trametes* were also found to hydrolyse carboxymethylcellulose and curdlan [a β-(1 → 3)-D-glucan], but none of the fractions was active towards a succinoglucan (a polysaccharide containing D-glucose and D-galactose) from *Alcaligenes faecalis*. An enzyme obtained from culture media of a *Flavobacterium* species grown on luteose as the sole source of carbon was active towards luteose and gentiobiose, but not towards the succinoglucan. However, growth of the organism on the succinoglucan produced an enzyme that was active towards the succinoglucan and a polysaccharide containing β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-linked D-glucose residues. Since no activity was formed when D-glucose was used as the source of carbon, it was concluded that the organism produces specific glucanases depending on the polysaccharide present in the culture medium.

One of the three fractions active against β-glucans secreted by protoplasts and intact cells of *Saccharomyces cerevisiae* appeared to consist of two

[261] J. J. Marshall, *Carbohydrate Res.*, 1973, **26**, 274.

enzymes exhibiting activity for pustulan (β-1,6-glucan), laminarin, periodate-oxidized laminarin, and 4-nitrophenyl β-D-glucopyranoside.[143] This fraction was distinct from another containing a non-specific *exo*-β-glucanase capable of hydrolysing both pustulan and laminarin.

Glucanases (Miscellaneous)

After removal of the various isoenzymes of α-amylase, human urine was still found to contain an α-glucanase that hydrolysed starch to D-glucose.[182]

A study of two α-glucosidase activities present in rabbit muscle has led to the conclusion that they represent two forms of a single enzyme possessing a pH optimum at 7.4.[131] As evidenced by its action on maltodextrins and panose, the enzyme possesses constitutive α-1,4- and α-1,6-glucanase activities, but it exhibited little activity towards glycogen. The enzyme is presumed to be an oligosaccharidase, which probably acts on glycogen in conjunction with α-amylase. The combined action of these enzymes may serve to control the structure of glycogen.

A considerable difference has been recognized in the activity of an 'α-oligo-glucosidase' from rabbit liver towards glycogen and amylopectin.[262] Potato amylopectin and its β-limit dextrin were hydrolysed nearly fifteen times faster than glycogen and a derived limit dextrin. These differences are considered to depend on the molecular weights and on the structures of the inner chains of the substrates.

The amylolytic activity present in the seeds of maize, oat, and wheat, and in the first leaves and coleoptiles of these plants was distinguishable from an α-amylase obtained from the endosperms of the germinated seeds.[194] During germination, the endosperms of oat and wheat seeds were found to contain amylolytic enzymes in addition to α-amylase.

Two α-glucosidases from rice seeds have been found to possess activities towards starch that are increased in the presence of potassium chloride, and uni- and bi-valent cations.[136] The activities of the enzymes towards maltose, however, were not affected by cations. In experiments with mixed substrates, liberation of D-[^{14}C]glucose from uniformly labelled [^{14}C]-maltose was not inhibited by the presence of starch, nor was the liberation of D-[^{14}C]glucose from uniformly labelled [^{14}C]starch inhibited by the presence of maltose. It was suggested that the α-glucosidases possess sites for hydrolysing maltose distinct from those responsible for hydrolysing starch. The α-glucosidases liberated D-glucose only from starch, which was presumed to be hydrolysed completely on extensive incubation.

Molecular-sieve chromatography of an extract from ungerminated seeds of rye has yielded a collection of enzymes that hydrolyse cellobiose, laminaribiose, cellodextrins, laminarin, and barley β-glucan.[137]

Heterogeneity of the activity towards the cell wall of yeast has been observed among the components of a glucanase obtained from an

[262] I. S. Lukomskaya and N. A. Ushakova, *Doklady Akad. Nauk S.S.S.R.*, 1973, **210**, 960.

Arthrobacter species.[263] The glucanase preparation had previously been shown to hydrolyse yeast glucans, with the concomitant liberation of laminaripentaose, but zymograms obtained by polyacrylamide-gel electrophoresis showed that it contained several proteinaceous components. Variations in the overall hydrolytic properties of the glucanase preparation were ascribed to the presence of variable proportions of individual components possessing different glycolytic activities. The purification of a glucanase from *Arthrobacter luteus* has been described, and the final preparation appeared to be homogeneous on Tiselius electrophoresis and ultracentrifugation.[264] A molecular weight of 2.05×10^4 was estimated from the sedimentation pattern. The lability to heat of the enzyme, which exhibited maximum activity at pH 7.5 and 35 °C, was investigated, and viable yeast cells, yeast glucan, certain laminarins, and heat-treated pachyman are effective substrates for the enzyme. Heat-treated pachyman yielded laminaripentaose only, whereas insoluble laminarin yielded laminaripentaose and oligosaccharides with DP > 7; soluble laminarin was not hydrolysed by the enzyme. Since neither curdlan nor pachyman was hydrolysed, it appears that the enzyme, in addition to being specific for long sequences of β-(1 → 3)-linked D-glucose residues, possesses some specificity for the arrangement of polysaccharide chains in the substrate.

The distribution of β-1,3-glucanase and chitinolytic and yeast-cell lytic enzymes among eighty-two strains of Basidiomycete species has been examined.[249] At pH 4 a number of strains of Aphyllophorales degraded both lyophilized and heated yeast (*Saccharomyces cerevisiae*) cells, whereas species of Agaricales exhibited no such activity. By contrast, when the activity was examined at pH 7, strains of Aphyllophorales were able to degrade only heat-treated cells, whereas those of Agaricales exhibited high activities against both forms of cells. In particular, Coprinaceae species of the Agaricales possessed high activity towards the heated yeast cells. Some species of Polyporaceae (*Gloeophyllum saepiarium*, *Trametes sanguinea*, and *Irpex lacteus*, *etc.*) secreted large quantities of β-1,3-glucanases into their culture media, and this activity seems to be produced by all Basidiomycete species. Since the lytic activity of the Coprinaceae towards yeast cells was exceptionally high compared with the β-1,3-glucanase and chitinolytic activities, it was concluded that an alkaline protease, having a pH optimum of 8.5—9.0 for its caseinolytic activity, is responsible.

Ion-exchange chromatography on DEAE-cellulose of the β-D-glucanases of a commercial enzyme preparation from a *Cytophaga* species has yielded three active fractions.[261] The unadsorbed fraction contained an *endo*-β-1,3-glucanase, but the second (adsorbed) fraction degraded carboxymethylcellulose and cellodextrins to oligosaccharides, and lichenin to 4-*O*-β-laminaribiosyl-D-glucose, a tetrasaccharide, and higher oligosaccharides. A molecular weight of 1.8×10^4 was estimated for the

[263] K. Doi, A. Doi, T. Ozaki, and T. Fukui, *J. Biochem.* (*Japan*), 1973, **73**, 667.
[264] K. Kitamura and Y. Yamamoto, *Arch. Biochem. Biophys.*, 1972, **153**, 403.

enzyme from gel-filtration data. The third (adsorbed) fraction possessed the characteristics of a laminarinase.

An enzyme obtained from culture media of a *Flavobacterium* species grown on a succinoglycan (a polysaccharide containing D-glucose, D-galactose, and succinic acid) from *Alcaligenes faecalis* as the sole source of carbon was active against the succinoglycan, the desuccinylated polysaccharide, and a polysaccharide consisting mainly of β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-D-glucose residues as well as a small proportion of β-(1 → 3)-linked D-galactose residues.[239] The enzyme showed slight activity towards laminarin. Use of luteose as the sole source of carbon produced an enzyme that was active against luteose [a β-(1 → 3)-linked D-glucan] and gentiobiose, but that was inactive against the succinoglycan and its desuccinylated derivative. No activity was detected when D-glucose was used as the sole source of carbon, and it was concluded that the bacterium produces different substrate-specific β-glucanases in response to the type of polysaccharide used in the growth medium.

Ion-exchange chromatography of the macromolecules secreted by protoplasts and intact cells of *Saccharomyces cerevisiae* revealed the presence of at least three fractions capable of hydrolysing laminarin.[143] The first fraction, which was not adsorbed by the column, appeared to consist of two enzymes exhibiting activity towards laminarin, 4-nitrophenyl β-D-glucopyranoside, and pustulan [a β-(1 → 6)-linked D-glucan], and a low, but detectable, activity towards periodate-oxidized laminarin. Another fraction possessed the substrate specificity of an *endo*-β-1,3-glucanase, whereas the third fraction was an unspecific *exo*-β-glucanase that hydrolysed the terminal residues of laminarin and pustulan. With either of these substrates or 4-nitrophenyl β-D-glucopyranoside, the enzyme exhibited maximum activity at pH 4.5—5.0. The properties of these extracellular β-glucanases were compared with those of similar enzymes occurring in protoplast lysates and cell-free extracts of intact cells.

Glucoamylases

In the hydrolysis of starch by glucoamylase (*exo*-1,4-α-glucosidase, amyloglucosidase, γ-amylase), extension of the theoretical approach to deal with a reaction more complex than first order has led to the conclusion that, under conditions generally employed, the hydrolysis is better carried out in a membrane reactor than in a solid-wall reactor.[265] Another advantage of the membrane reactor is that it separates the enzyme from the D-glucose produced, thereby allowing re-cycling and re-use of the enzyme.

Extracts of biopsy specimens of skeletal muscle obtained from normal subjects, cases of β-glucosidase-maltase deficiency in adults, and a case of myophosphorylase deficiency were found to contain glucoamylase activity.[129]

[265] G. P. Closset, Y. T. Shah, and J. T. Cobb, *Biotechnol. and Bioeng.*, 1973, **15**, 441.

Human intestinal glucoamylase has been purified to near homogeneity by means of polyacrylamide-gel electrophoresis.[266] At least two isoenzymes, possessing identical catalytic properties, were found. Linear oligosaccharides containing nine α-(1 → 4)-linked D-glucose residues possessed the greatest affinity for the active site of the enzyme. The purified enzyme hydrolysed both α-(1 → 4)- and α-(1 → 6)-linkages, but linear polymers of D-glucose containing α-(1 → 4)-linkages were hydrolysed more rapidly than those containing α-(1 → 6)-linkages. The activities of the enzyme against both types of linkage were simultaneously removed on heating, and no β-fructofuranosidase activity could be detected. Thus, hydrolysis of α-(1 → 6)-linkages is not due to contamination by other intestinal enzymes. The purified glucoamylase contains 32—38% carbohydrate consisting of galactose (4.5), mannose (5.1), fucose (17.9), xylose (0.3), 2-amino-2-deoxygalactose (1.5), and 2-amino-2-deoxyglucose (4.7%). The absence of sialic acid and phosphate ester (*i.e.* membrane components) was noted. Equilibrium sedimentation showed that the enzyme possesses a molecular weight of 2.1×10^5 and a partial specific volume of 0.684.

The role of carboxylic acid groups in the action of glucoamylase from *Aspergillus niger* has been investigated by reaction of the enzyme with glycine methyl ester in the presence of a water-soluble carbodi-imide.[267] The loss of activity sustained by this reaction in the presence of maltose was considerably less than that in the absence of maltose, from which it was concluded that two or three carboxylic acid groups are present at the active site of the enzyme, and that modification thereof causes loss of activity. Approximately thirty-six carboxylic acid groups appear to be exposed generally over the surface of the intact molecule, and it is the reaction of these groups that causes the initial, rapid loss of about 20% of the enzymic activity on derivatization. The loss of activity is not unexpected, since reaction of the carboxylic acid groups results in the removal of one negative charge per residue and is liable to produce a marked change of conformation. The effect of glucoamylase from *A. niger* on the release of insulin in mice has been investigated.[268]

Glucoamylase was among six glycosidases and glycanases that have been detected in fractionations of the enzymes from a Basidiomycete species by ion-exchange chromatography.[139]

The glucoamylase from *Rhizopus delemar* has been found to catalyse the hydrolysis of phenyl α-D-glucopyranoside, although the rate at which it does so is much slower than that for phenyl α-maltoside.[269] The K_m values and molecular activities (k_0) were determined for the glucoamylase-catalysed hydrolyses of 4-nitrophenyl, phenyl, 4-methylphenyl, and

[266] J. J. Kelly and D. H. Alpers, *Biochim. Biophys. Acta*, 1973, **315**, 113.
[267] C. J. Gray and M. E. Jolley, *F.E.B.S. Letters*, 1973, **29**, 197.
[268] I. Lundquist, *Hormone Metab. Res.*, 1972, **4**, 341.
[269] N. Suetsugu, E. Hirooka, H. Yasui, K. Hiromi, and S. Ono, *J. Biochem.* (*Japan*), 1973, **73**, 1223.

4-t-butyl α-D-glucopyranosides and the corresponding α-maltosides at pH 4.5 and 25 °C. The effect of the aglycone on the K_m value was marked for both types of substrate, and there was a slight effect on k_0 for α-D-glucopyranosides, whereas k_0 for the maltosides was unaffected. Comparison of the values of K_m and k_0 for a particular D-glucopyranoside with those of the corresponding maltoside showed that the K_m value was ten times larger and that k_0 was five hundred to a thousand times smaller. This remarkable difference in k_0 for the two series of substrates cannot be explained on the basis of the chemical nature of the bond to be hydrolysed, but it could be reasonably accounted for in terms of the statistical weight of productive and non-productive complexes that are determined by the subsite affinities of the enzyme. The dependence of K_m and k_0 on the DP of malto-oligosaccharide substrates in hydrolyses catalysed by glucoamylase from *R. delemar* has been studied at pH 4.5 and 25 °C for DPs of 2—16.[270] The results were analysed to evaluate the subsite affinities (A_i) and the intrinsic rate constant for hydrolysis of substrate linkages in a productive complex (k_{int}) according to a previously reported theory, which assumes the independency of k_{int} of the DP and the additivity of A_i. Values of K_m and k_0, calculated with the aid of the values determined for A_i and k_{int}, were in good agreement with values obtained experimentally for DPs of 2—7, thus confirming the validity of the theory.

Immobilized forms of glucoamylase have been produced by covalent attachment of forms of the enzyme to beads of diazotized poly-(aminostyrene),[271] and to cellulose cyclic imidocarbonate,[272] cellulose bromo- and iodo-acetates,[273] and titanium chelates of poly-(4- and 5-acrylamidosalicylic acids).[223] In the latter case, the derivative withstood extensive washing and could be used continuously in a column. Derivatives of porous glass have also been used to support an insoluble form of glucoamylase, the enzyme being coupled covalently either to azo-groups or with the aid of glutaraldehyde.[274] Glucoamylase has been immobilized by entrapping processes; for example, by *X*-ray-induced polymerization of acrylamide in the presence of the enzyme,[81] by photopolymerization of aqueous solutions containing the enzyme, acrylamide, a cross-linking monomer (allyl glycidyl ether), an enzyme-linking monomer (glycidyl acrylate), and an oxygen scavenger,[274] and by wet-spinning an emulsion of an aqueous solution of the enzyme with a solution of cellulose acetate in methylene chloride.[275] The properties and possible applications of the insoluble glucoamylase derivatives were described. Glucoamylase has also been coupled to insulin, with the aid of glutaraldehyde, to give a

[270] K. Hiromi, Y. Nitta, C. Numata, and S. Ono, *Biochim. Biophys. Acta*, 1973, **302**, 362.
[271] W. M. Ledingham and M. do Socorro Santos Ferreira, *Carbohydrate Res.*, 1973, **30**, 196.
[272] H. Maeda and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1839.
[273] H. Maeda and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1581.
[274] D. R. Marsh, Y. Y. Lee, and S. T. Tsao, *Biotechnol. and Bioeng.*, 1973, **15**, 483.
[275] C. Corno, G. Galli, F. Morisi, M. Bettone, and A. Stopponi, *Stärke*, 1972, **24**, 420.

soluble, immobilized derivative that was used in a fluorometric immunoassay of the enzyme.[276]

exo-β-1,3-Glucosidases

Changes in the levels of various β-glucan hydrolases, including *exo*-β-1,3-glucanase activity, in the second internode of the stems of developing oat plants have been studied.[134] Concurrent changes in the non-cellulosic β-glucans contained in the corresponding total hemicelluloses were also investigated. Possible relationships between the observed changes and the growth and development of the plant tissues were discussed.

A β-glucanase has been purified from culture media of *Rhizopus niveus* by treatment with calcium acetate, fractionation with poly(ethylene glycol), batch and column ion-exchange chromatography, and gel filtration.[277] The final preparation, which was homogeneous on disc electrophoresis on polyacrylamide gels and on ultracentrifugation, hydrolysed laminarin to give D-glucose as the only product. Enzymic activity was optimal at pH 5.5 and 60 °C, and was inhibited by mercury(II) ions.

exo-β-1,4-Glucosidases

Changes in the levels of various β-glucanases, including *exo*-β-1,4-glucanase activity, in the second internode of the stems of developing oat plants have been studied.[134] Concurrent changes in the non-cellulosic β-glucans contained in the corresponding total hemicelluloses were also investigated. Possible relationships between the growth and development of the plant tissues and the observed changes were discussed.

exo-α-1,6-Glucosidases

A preparation of α-D-glucanase isolated from a soil bacterium has been shown to degrade dextran principally to D-glucose, but it was apparently unable to attack certain non-(1 → 6)-linkages.[252] The initial attack on dextran appeared to be of the *endo* type, but attack on linear isomaltodextrins appeared to be of the *exo* type, with one D-glucose unit at a time being liberated from the non-reducing end of the substrate. The pH and temperature optima and the thermal stability of the enzyme were investigated.

exo-α-1,6-Glucosidase from *Streptococcus mitis* has been used to confirm the terminal position of the D-[^{14}C]glucose residues of a series of labelled isomalto-oligosaccharides, prepared by incubation of dextran-sucrase with [^{14}C]sucrose in the presence of an excess of unlabelled isomalto-oligosaccharides as alternative acceptors.[253]

Hyaluronidases and Hyaluronate Lyases

The levels of tissue hyaluronidase and bacterial hyaluronate lyase (hyaluronidase) in whole-mouth saliva samples have been measured for

[276] E. Ishikawa, *J. Biochem.* (*Japan*), 1973, **73**, 1319.
[277] H. Horitsu, T. Satake, and M. Tomoyeda, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1007.

males, for normal females taking and not taking oral contraceptives, and for females with irregular menstrual cycles.[278] The changes in activities that occur throughout the menstrual cycle were determined.

Determination of the anomeric configuration of the products resulting from the action of a carbohydrase has been based on the observation that the sign of the accumulation rate of the P_1-anomer in the process

$$\text{substrate} \xrightarrow{\text{enzyme}} \text{anomer } P_1 \rightleftharpoons \text{anomer } P_2$$

can change under certain conditions, whereas that of the P_2-anomer remains constant.[21] Using this method, hydrolysis of hyaluronic acid by testicular hyaluronidase was found to involve cleavage of the 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-bond with retention of the anomeric configuration. The accuracy of the determination was confirmed by examination of two enzyme systems for which the anomeric configurations of the products had been determined by other methods.

A fresh preparation of bovine testicular hyaluronidase, which was homogeneous on polyacrylamide-gel electrophoresis and by sedimentation, gave two protein bands (molecular weights 6.0×10^4 and 1.4×10^4) on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate.[279] On extended incubation in the presence of this detergent, only the material of lower molecular weight was detectable. Analogous electrophoresis of the succinylated enzyme gave closely similar results, except that the enzyme dissociated more readily to give components having molecular weights of 2.8×10^4 and 1.4×10^4. Sedimentation analysis of the natural enzyme at various pHs gave values of 3.76 *S* at pH 5.7, falling to 2.06 *S* at pH 11.3; similar analysis of the succinylated enzyme gave a value of 2.57 *S* at pH 5.7, but the molecule appeared to be heterogeneous at higher pH values: for example, 6.2 *S* and 4.3 *S* at pH 10. From these data it was concluded that the enzyme possesses a quaternary structure and consists of four subunits of molecular weight 1.4×10^4. A molecular weight of 5.5×10^4 was calculated for the native enzyme, and its $S_{20,w}$ value was estimated to be 4.17 *S*. The hyaluronidase exhibited a diffusion constant of 6.8×10^{-7} cm^2 s^{-1} and was found to contain 3.5% carbohydrate.

Intraperitoneal administration of deoxycorticosterone to rabbits caused the appearance of a hyaluronidase in the vitreous body of the eye.[100] It is supposed that this and other enzymes participate in the mechanism whereby the intraocular pressure is increased by the action of deoxycorticosterone.

A summary of the chemical structure and biological action of the components of bee venom has drawn attention to differences between

[278] R. E. S. Prout and R. M. Hopps, *J. Periodontal Res.*, 1973, **8**, 86.
[279] A. Y. Khorlin, I. V. Vikha, and A. N. Milishnikov, *F.E.B.S. Letters*, 1973, **31**, 107.

this endogenous hyaluronidase and mammalian hyaluronidase.[280] The hyaluronidase of bee venom has been purified by gel filtration and ion-exchange chromatography on QAE-Sephadex.[281] Of a number of metal ions tested, solutions of iron and aluminium salts were found to be better activators of the enzyme than either calcium or magnesium ions. The enzyme was inactivated by H_4edta, owing to the exposure of SH groups.

Hyaluronidase has been shown to be a major constituent of the venom of the Tarantula spider (*Dugesiella hentzi*) using a turbidimetric method and a hyaluronic acid–hyaluronic acid–albumin complex as substrate.[282] The enzyme could be purified by either gel filtration or isoelectric focusing, and possesses a molecular weight of 3.96×10^4 and a p*I* of 6.9; it exhibited maximum activity at pH 3.5 and was stable for several months in the frozen state. Products of low molecular weight resulted from sustained action of the enzyme on the aforementioned substrate. Venom of the poisonous Brown Recluse Spider (*Loxosceles reclusa*) was found to contain a hyaluronidase, which could be obtained in monophoretic form by gel filtration and polyacrylamide-gel electrophoresis.[283] The purified enzyme exhibited optimum activity in the range pH 5.0—6.6, and showed reduced (20—30%) activity towards chondroitin 4- and 6-sulphates and dermatan sulphate. The enzyme was partly inhibited by iron(III) and copper(II) ions, and rabbit anti-venom inhibited the hyaluronidase *in vitro*. The incorporation of [^{14}C]leucine into the spider venom was used in a separation of the hyaluronidase and dermonecrotic activities thereof. Polyacrylamide-gel electrophoresis of the purified enzyme in the presence of sodium dodecyl sulphate revealed the existence of two components with estimated molecular weights of 3.3×10^4 and 6.3×10^4.

Hyaluronate lyase from *Staphylococcus aureus* has been purified by fractional precipitation with ammonium sulphate and acetone, gel filtration, and ion-exchange chromatography.[284] Crystallization gave a product that was chromatographically and electrophoretically homogeneous. During passage through a cation-exchange column, two minor peaks of hyaluronate lyase activity were also recognized. The purified enzyme was shown to contain protein and carbohydrate in the ratio of 5 : 1, the carbohydrate portion consisting of galactose, glucose, mannose, 2-amino-2-deoxygalactose, and 2-amino-2-deoxy-glucose or -mannose (molar ratios 2 : 6 : 12 : 1 : 1, respectively). Quantitative amino-acid analyses showed a predominance of the basic amino-acids lysine and histidine.

[280] E. Habermann, *Angew. Chem. Internat. Edn.*, 1973, **12**, 83.
[281] M. A. Krysteva, B. K. Mesrob, C. P. Ivanov, and S. Shkenderov, *Doklady Bolg. Akad. Nauk*, 1973, **26**, 917.
[282] F. L. Schanbacher, C. K. Lee, I. B. Wilson, D. E. Howell, and G. V. Odell, *Comp. Biochem. Physiol.*, 1973, **44B**, 389.
[283] R. P. Wright, K. D. Elgert, B. J. Campbell, and J. T. Barrett, *Arch. Biochem. Biophys.*, 1973, **159**, 415.
[284] G. S. Rautela and C. Abramson, *Arch. Biochem. Biophys.*, 1973, **158**, 687.

Inulinases (Inulases)

The inulinase and β-fructofuranosidase activities of an enzyme from *Saccharomyces fragilis* have been studied.[78]

Isoamylases

The specific activity of isoamylase from a *Pseudomonas* species has been determined in terms of the cleavage of glycosidic bonds per minute per milligram of enzyme at substrate concentrations of 2%, and the values so obtained were compared with those for pullulanase from an *Aerobacter* species.[285] Values of 110—280 and 3—5 μmol for amylopectins, 110—280 and 0.5—1.2 μmol for glycogens, and 1.1 and 53 μmol for pullulan, respectively, were obtained for the two enzymes. K_m Values for the actions of the isoamylase and pullulanase were found to be $1—2 \times 10^{-4}$ and $8—10 \times 10^{-3}$ for amylopectin, $1—2 \times 10^{-4}$ and $2—5 \times 10^{-2}$ for glycogen, and 2×10^{-3} and 1.7×10^{-5} g ml^{-1} for pullulan, respectively. The molecular activity (the number of equivalents of a group transformed per minute per mole of enzyme at the optimum concentration of substrate) of the isoamylase was $9.5—26.7 \times 10^3$ for amylopectins and glycogens, but only 110 for pullulan.

Isopullulanases

An enzyme that liberated a large proportion of isopanose (6-α-maltosyl-D-glucose) and a small proportion of a tetrasaccharide from pullulan has been purified from *Aspergillus niger* by ion-exchange chromatography and gel filtration.[286] The enzyme exhibited a pH optimum of 3.0—3.5 and a temperature optimum of 40 °C at pH 3.5; it was stable to heat in the range pH 2—8. The purified enzyme attacked (1 → 4)-α-D-glucosidic linkages adjacent to (1 → 6)-α-D-glucosidic linkages at the reducing end of pullulan, 6^3-α-D-glucopyranosylmaltotriose, 6^2-α-maltosylmaltose, and panose (6^2-α-D-glucopyranosylmaltose) to liberate isopanose, isomaltose and maltose, isopanose and D-glucose, and isomaltose and D-glucose, respectively. The molecular weight (by gel filtration) of the isopullulanase was estimated to be 7.4×10^4.

Laminarinases

Molecular-sieve chromatography of an extract from ungerminated seeds of rye has revealed the presence of enzymes that hydrolyse laminarin, cellobiose, cellodextrin, barley β-glucan, and laminaribiose.[137] Laminarinase from a *Cytophaga* species has been purified to a high degree of homogeneity on DEAE-cellulose.[232] Since the use of DEAE-Sephadex gave a poor separation of the active and inactive proteins, fractionation on the cellulose

[285] K. Yokobayashi, H. Akai, T. Sugimoto, M. Hirao, K. Sugimoto, and T. Harada, *Biochim. Biophys. Acta*, 1973, **293**, 197.
[286] Y. Sakano, M. Higuchi, and T. Kobayashi, *Arch. Biochem. Biophys.*, 1972, **153**, 180.

derivative is indicated to be a form of affinity chromatography. Fractionation of a commercial enzyme preparation from a *Cytophaga* species on DEAE-cellulose yielded three fractions containing β-glucanases.[261] A fraction not adsorbed by the column acted only on laminarin, and appeared to be an *endo*-β-1,3-glucanase. Another (adsorbed) fraction was active towards carboxymethylcellulose, cellodextrins, and lichenin, whereas a third fraction (eluted by acid) acted on laminarin to give laminaribiose and D-glucose, and on lichenin to give 3-*O*-β-cellobiosyl-D-glucose, laminaribiose, and D-glucose. Further purification of this laminarinase by gel filtration yielded a form (molecular weight 8×10^3) that was homogeneous on polyacrylamide-gel electrophoresis.

Limit Dextrinases

Limit dextrinase has been purified from malted sorghum by chromatography on a starch–Celite column (to remove α-amylase activity) and gel filtration.[287] Limit dextrinases have also been purified by ion-exchange chromatography and gel filtration of extracts from ungerminated seeds of oats and rice. The rates of hydrolysis of amylopectin by three limit dextrinases were found to be only 20% of those measured for amylopectin β-limit dextrin under similar conditions; the actions of the enzymes on glycogen and its β-limit dextrin were also examined. Factors controlling the specificity of the enzymes and the possible significance *in vivo* of the results were discussed. The results also support an earlier view that the specificity of debranching enzymes may be controlled partly by the length of the side-chain released and partly by the relevant position of attachment of the side-chain.

Lysozymes

The multiple, acid–base catalysis that occurs at the active sites of lysozyme has been discussed in a book on catalysis and the actions of enzymes.[3] In a review of physical-organic models for the mechanism of action of lysozyme, the following aspects were covered: the characteristics of the enzymic reaction, physicochemical studies with model systems, and an evaluation of possible enzymic mechanisms.[288] Applications of Raman spectroscopy to biological molecules, including lysozyme, have been reviewed.[289]

A simple, colorimetric method for the determination of lysozyme activity has utilized as substrate glycol-chitin labelled with Remazol-brillant Blue R.[290] Coloured material is released by the enzyme and, after the reaction has proceeded for the required time, the residual substrate is precipitated with acidified ethanol.

[287] G. Dunn, D. G. Hardie, and D. J. Manners, *Biochem. J.*, 1973, **133**, 413.
[288] B. M. Dunn and T. C. Bruice, *Adv. Enzymol.*, 1973, **37**, 1.
[289] J. L. Koenig, *Macromol. Rev.*, 1972, **6**, 59.
[290] N. Yamasaki, T. Tsujita, and M. Takakuwa, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1507.

1-Glycopyranosylimidazoles have been found to act as effective inhibitors of glycosidases, but 1-β-D-glucopyranosylimidazole was unable to inhibit the degradation of *Micrococcus lysodeikticus* cells by lysozyme.[24] This result is not considered out of place, since lysozyme requires the presence of either amino- or acetamido-groups in the substrate to manifest its action. However, 1-α-D-glucopyranosylimidazole behaved as a non-competitive inhibitor of lysozyme.

The presence of lipopolysaccharides on the surface of *Escherichia coli* cells has been reported to prevent lysozyme from penetrating into the bacterial cells.[291]

A number of applications of chitin-coated cellulose as an adsorbent for lysozyme-like enzymes have been reported.[248] Affinity chromatography of saliva on this matrix yielded a fraction possessing some activity towards bacterial substrates, but which displayed significant activity towards glycol-chitin. Neither activity was present in a non-specific fraction eluted from the column. Similar results were obtained with an extract of taro. In both cases, the isolated enzymes are considered to be lysozymes. Fractionation of an extract of yam on chitin-coated cellulose yielded a chitinase possessing no lytic activity.

The results of fluorometric and spectrophotometric titrations of lysozyme from humans and its complexes with oligomers of 2-acetamido-2-deoxy-D-glucose have been compared with those from a number of parallel studies on hen egg-white lysozyme.[292] Spectrophotometric titration and difference spectra in alkali of a complex of human lysozyme and [(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-]$_3$-2-acetamido-2-deoxy-D-glucose showed that the pK_{int} value of the tyrosine-63 (-62) residue is lowered from 10.5 to 10.0 when the inhibitor is bound. The fluorescence of the enzyme from humans has a lower quantum efficiency than that of the avian enzyme and maximum emission occurs at a shorter wavelength. The spectrofluorometric titration curves of the two enzymes were very similar, whereas those of the complexes with inhibitors did not exhibit the same dependence on pH in the acid region. Below pH 5.5, the fluorescence of the complex of hen egg-white lysozyme and [(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-]$_2$-2-acetamido-2-deoxy-D-glucose was strongly quenched, whereas the fluorescence of the corresponding complex with the enzyme from humans was as intense as that of the free enzyme. The marked enhancement of fluorescence occurring at pH 7.5 on binding of inhibitors was used to determine the association constants of a series of [(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-]$_{1-4}$-2-acetamido-2-deoxy-D-glucoses. A difference was recognized in the free energy of binding of the two enzymes, which could be related to substitution of tryptophan-62 of the avian form by tyrosine-63 (-62) in the human form.

[291] S. Tamaki and M. Matsuhashi, *J. Bacteriol.*, 1973, **114**, 453.
[292] R. S. Mulvey, R. J. Gualtieri, and S. Beychok, *Biochemistry*, 1973, **12**, 2683.

The substrate specificities of lysozymes from human saliva, the whites of duck, hen, and quail eggs, and turnip have been investigated.[293] Reducing end-groups and oligosaccharides were liberated from bacterial cell walls and glycol- and carboxymethyl-chitins by the enzymes, which showed similar specificities towards these substrates. Two lysozyme fractions could be isolated from turnips and were separated by ion-exchange chromatography and gel filtration.

Comparative studies of the lysozymes of leucocytes from normal subjects and patients with chronic myelogenous leukaemia have been reported.[294] Ion-exchange chromatography revealed the presence of only one form of the enzyme in normal leucocytes, but of two active forms in the diseased cells. Studies of the molecular weights, amino-acid compositions, u.v. spectra, electrophoretic behaviours, *N*-terminal amino-acids, and specificities against *NN'N''N'''N''''*-penta-acetylchitopentaose showed that the latter two enzymes did not differ in all respects. However, their behaviour on re-chromatography on ion-exchange resins suggested a difference in the amide-nitrogen contents. Lysozyme in blood from human umbilical cord was found to resemble the enzyme from normal leucocytes.

Starch-block electrophoresis and chromatography on DEAE-cellulose of human-serum lysozyme afforded two active components; a plate assay was used to follow the activity during fractionation.[295] The presence of two types of activity could be explained in terms of either isoenzymes or one form being a complex version of the other. The lysozymes in sera of cases of overwhelming infections, acute renal failure, monocytic leukaemia, monomyelocytic leukaemia, and other conditions were investigated.

The interactions of 2-acetamido-2-deoxy-D-glucose, its methyl α- and β-glycosides, and [(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-]$_{1 \text{ or } 2}$-2-acetamido-2-deoxy-D-glucose with the lysozymes from human leukaemic urine and hen-egg white have been studied by c.d.[296] The association constants obtained for the avian enzyme were found to be in good agreement with those reported by others, and were larger than those obtained for the urinary lysozyme. The free energy for binding sugar residues at each subsite of the substrate-binding site of urinary lysozyme was compared with those for the lysozyme from hen egg-white, and was discussed on the basis of measurements obtained by *X*-ray crystallography.

The association of endogenous lysozyme with other molecules in rat liver, spleen, and kidney has been investigated.[297]

Single crystals suitable for high-resolution *X*-ray diffraction studies have been obtained from the lysozyme of duck egg-white by incorporation of suitable heavy atoms [*e.g.* mercury(II)].[298] The unit-cell dimensions were

[293] S. Hara and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 993.
[294] J.-P. Périn and P. Jollès, *Clinica Chim. Acta*, 1972, **42**, 77.
[295] R. E. Noble and H. H. Fudenberg, *Enzyme*, 1972/3, **14**, 55.
[296] S. Kuramitsu, K. Ikeda, K. Hamaguchi, S. Miwa, and T. Nishina, *J. Biochem.* (*Japan*), 1972, **72**, 1109.
[297] R. Raghunathan and S. Gurnani, *Indian J. Biochem. Biophys.*, 1972, **9**, 166.
[298] J. Berthou, A. Laurent, and P. Jollès, *J. Mol. Biol.*, 1972, **71**, 815.

found to be $a = 2.85$, $b = 6.62$, $c = 3.2$ nm, and $\beta = 113°$. The dispositions of the disulphide bonds of duck egg-white lysozyme II were established in detail by a study of the cystine-containing peptides released on enzymic digestion.[299] Comparison of a number of optical properties of duck egg-white lysozymes II and III suggested that the same disposition of disulphide bonds occurs in both.

Isolation of lysozyme of 95% purity from hen egg-white has been achieved by affinity chromatography; the affinant was prepared by treating cellulose with alkali-chitin and, in the form of a packed column, could be used repeatedly with good retention of stability.[300] The column adsorbed the enzyme specifically and desorption was effected under mild conditions using acetic acid.

The viscosities of dilute solutions of hen egg-white lysozyme have been measured in the pH range 1.4—12.7 at various concentrations of sodium chloride.[301] Below the isoelectric point (pH 11) of the enzyme, the viscosity of the solution decreased with increasing pH owing to the diminishing electroviscous effect. The viscosity reached a minimum at pH 11 and thereafter increased at higher pH values owing to coagulation. The electroviscous effect was found to be depressed by increasing the concentration of small ions. These effects were discussed in relation to Booth's theory, and partial agreement between theory and experiment was obtained; discrepancies between the two were attributed to the non-spherical distribution of charges on the protein. The molecular volume of lysozyme derived from Einstein's equation (by extrapolation of the reduced viscosity to a sufficiently high ionic concentration) compared well with that in the crystal.

The pH-dependence between pH 4 and 12 of the ellipticity of the negative band at 305 nm in the c.d. spectrum of hen egg-white lysozyme has been examined in detail.[302] This dependence could be ascribed to two components: one, with an intrinsic pH value of 6.9, representing the interaction of tryptophan-108 and glutamic acid-35, and the other, with an intrinsic pK value of 10.2, representing the ionization of a tyrosyl residue. Esterification of the enzyme with *N*-acetylimidazole resulted in a change in the c.d. spectrum, which could not be completely reversed on deacetylation. The deacetylated lysozyme showed a pH-dependence of the ellipticity at 305 nm similar to that found for the untreated enzyme, but with a shift of 0.5 units to higher pH.

An investigation of the influence of urea on the crystallization and polymorphism of hen egg-white lysozyme has shown that if urea does not prevent crystallization, it is not a good additive.[303] In almost all cases,

[299] J. Hermann, J. Jollès, and P. Jollès, *Arch. Biochem. Biophys.*, 1973, **158**, 355.
[300] T. Imoto and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 465.
[301] M. Komatsubara, K. Suzuki, H. Nakajima, and Y. Wada, *Biopolymers*, 1973, **12**, 1741.
[302] Y. Nakae, K. Ikeda, T. Azuma, and K. Hamaguchi, *J. Biochem. (Japan)*, 1972, **72**, 1155.
[303] J. Berthou and P. Jollès, *F.E.B.S. Letters*, 1973, **31**, 189.

urea delayed growth at high and low concentrations; below 25 °C, A-type crystals were obtained, but above this temperature urea induced changes in the B-form, so that the crystals were no longer isomorphous. At higher concentrations of urea, lysozyme crystallized in a new orthorhombic form.

The oligosaccharides (3)—(5), containing residues of 2-amino-2-deoxy-D-glucose, have been obtained by the action of *N*-acetylmuramoyl-L-alanine amidase on a partially *N*-acetylated peptidoglycan from *Bacillus cereus*.[304]

D-Glc*p*NH_2-Mur*N*Ac-D-Glc*p*NAc-Mur*N*Ac

(3)

D-Glc*p*NH_2-Mur*N*Ac-D-Glc*p*NH_2-Mur*N*Ac-D-Glc*p*NAc-Mur*N*Ac

(4)

D-Glc*N*Ac-Mur*N*Ac-D-Glc*p*NH_2-Mur*N*Ac-D-Glc*p*NAc-Mur*N*Ac

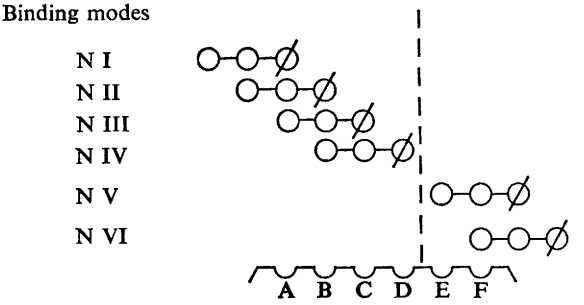
(5)

Hen egg-white lysozyme was unable to hydrolyse oligosaccharides (3) and (4), whereas it cleaved hexasaccharide (5) into (3) and 2-acetamido-2-deoxy-D-glucopyranosyl-*N*-acetylmuramic acid. These findings confirmed that there is a requirement for the acetamido-group of 2-acetamido-2-deoxy-D-glucose in the interaction with subsite C in lysozyme-catalysed reactions. The action of hen egg-white lysozyme on the alga *Anabaena ambigua* has been investigated.[305]

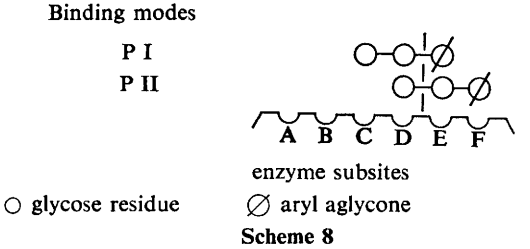
Using absorbance and fluorescence procedures, the association of hen egg-white lysozyme with *NN'N"N"'*-tetra-acetylchitotetraose, *NN'*-diacetylchitobiose, and 2-acetamido-2-deoxy-D-glucose at 10—80 °C and pH 0—9 has been studied.[306] The difference spectrum of the complex-free protein varied with pH and temperature. Analysis of the association data indicated that binding of the trimer perturbs the p*K* of ionization of three amino-acid residues in the protein chain, *viz.* glutamine-35, aspartic acid-66, and aspartic acid-101. *NN'N"N"'*-Tetra-acetylchitotetraose has been found to bind to the hen lysozyme in a different way from the corresponding trisaccharide.[307] The apparent enthalpy of binding of the tetrasaccharide is 11.7 kJ mol^{-1} less negative than that of the trisaccharide. Because of enthalpy–entropy compensation, the free energies of binding of tetra- and tri-saccharides differ only slightly (by 1.3 kJ mol^{-1}). Consideration of these results, in conjunction with other properties of the enzyme, suggested that there are two tetrasaccharide–lysozyme complexes, one of which has the characteristics of the trisaccharide–lysozyme complex. The corresponding penta- and hexa-saccharides are bound to the enzyme in an analogous way to the tetrasaccharide.

[304] H. Hayashi, K. Amano, Y. Araki, and E. Ito, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 641.
[305] N. C. Bhattacharya and E. R. S. Talpasayl, *Arkiv Mikrobiol.*, 1973, **90**, 157.
[306] S. K. Banerjee and J. A. Rupley, *J. Biol. Chem.*, 1973, **248**, 2117.
[307] S. K. Banerjee and J. A. Rupley, *Arch. Biochem. Biophys.*, 1973, **155**, 19.

Various u.v. and ^1H n.m.r. spectroscopic methods have been used to examine the nature and extent of non-productive interactions of hen egg-white lysozyme with *NN'*-diacetylchitobiose, *NN'N"*-triacetylchitotriose, 4-methylphenyl and 4-nitrophenyl *NN'*-diacetyl-β-chitobiosides, and 4-methylphenyl and 4-nitrophenyl *NN'*-diacetyl-1-thio-β-chitobiosides.[308] In the non-productive binding mode, two glycose residues are considered to interact with subsites B and C (see Scheme 7). Information concerning productive interactions was deduced indirectly from the rates of liberation of phenols and from product analyses. Binding modes that do not place the two glycose residues at either subsites C and D or subsites D and E are non-productive, and the involvement of one of these pairs of subsites is essential for productive binding (Scheme 8). The contributions of the aryl aglycones to non-productive and productive interactions



Scheme 7



Scheme 8

suggested that there is some flexibility of substrate specificity in reactions catalysed by lysozyme.

The modes of interaction between hen egg-white lysozyme and 4-methoxyphenyl *NN'*-diacetyl-β-chitobioside have been derived from a

[308] R. Otson, C. Reyes-Zamora, J. Y. Tang, and C. S. Tsai, *Canad. J. Biochem.*, 1973, **51**, 1.

^{1}H n.m.r. study of the response of the substrate to temperature.[309] At 32 °C, the methyl protons (*A*) of the acetamido-group proximal to the aryl glycone of the substrate underwent an upfield shift and line broadening, whereas those (*B*) distal from the aryl aglycone did not exhibit an isotropic shift. However, on raising the temperature, protons *B* gradually shifted upfield, whereas protons *A* were restored to their original position. These observations were related to a temperature-dependent transition of the lysozyme–chitobioside complex. Thus, at 32 °C, 4-methoxyphenyl *NN'*-diacetyl-β-chitobioside interacted with lysozyme by placing the pyranose ring proximal to the aryl glycone at subsite C, indicated by an upfield shift and line broadening of the methyl protons *A*. This interaction yielded a non-productive complex. At 65 °C, acetamido-methyl protons *B* underwent an upfield shift and line broadening, implying that the pyranose ring distal from the aryl aglycone interacted with subsite C. The rate of lysozyme-catalysed hydrolysis of the chitobioside at 35 °C displayed an induction period that disappeared at 55 °C. These observations accord with the preferential, productive interaction of the substrate with lysozyme at high temperatures (as deduced from n.m.r. studies), and attention was drawn to the fact that the thermodynamically unfavourable interaction between *NN'*-diacetyl-β-chitobioside and the enzyme may be overcome by raising the temperature.

Interactions of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, methyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside, acetamides, and alcohols with hen egg-white lysozyme have been shown to induce changes in the c.d. bands of tryptophanyl residues of the protein.[310] The effects on the c.d. spectrum and the binding energy for methyl 2-acetamido-2,6-dideoxy-α-D-glucopyranoside were essentially the same as those for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. This indicated that the hydrogen bond between O-6 of 2-acetamido-2-deoxy-β-D-glucopyranose and methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides and the indole imino-group of tryptophan-62 is not important for the binding of these sugars. Acetamides and alcohols were shown to interact with lysozyme at the substrate-binding subsite C, but the binding of alcohols was stronger than that of *N*-substituted acetamides having the same alkyl chain-length. The binding constants for *N*-(2-hydroxyalkyl)acetamides were found to be larger than those of the corresponding *N*-alkylacetamides. As assessed by n.m.r. spectroscopy, association of the methyl 2-acetamido-6-*O*-(*N*-methylisonicotinylium)-2-deoxy-β-D-glucopyranoside ion (from the corresponding iodide or chloride) with hen egg-white lysozyme caused chemical shifts of the acetamido- and glycosidic methyl groups comparable with those observed with the monosaccharide inhibitor methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[311] Thus, the binding modes of the two

[309] C. S. Tsai, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 205.
[310] S. Kuramitsu, K. Ikeda, and K. Hamaguchi, *J. Biochem.* (*Japan*), 1973, **74**, 143.
[311] J. Verhoeven and R. Schwyzer, *Helv. Chim. Acta*, 1972, **55**, 2572.

glycosides to the enzyme appear to be similar in solution. Moreover, a charge-transfer interaction of the pyridinium–indole type was observed spectrophotometrically, indicating that the complex of ionic glycoside and lysozyme in solution is similar to those of mono- and oligo-saccharide inhibitors in crystals. Complexation was reported to place O-6 of the ligand near to the tryptophanyl-62 residue of the enzyme.

An extract of *Micrococcus lysodeikticus* cells, which had been *N*-acetylated and partially *O*-acetylated, has been used as a substrate in an examination of the steric relationships between the active site of hen egg-white lysozyme and bound substrates, with particular reference to subsites C and D.[312] It was found that 2-acetamido-2-deoxy-6-*O*-methyl-D-glucosyl residues could fit easily into subsite C, whereas 2-acetamido-2-deoxy-3-*O*-methyl- and -3,6-di-*O*-methyl-D-glucose could not. 6-*O*-Methyl-*N*-acetylmuramic acid did not fit readily into subsite D.

Measurement of the c.d. spectra has proved useful in assessing the interaction of such bivalent cations as Mn^{2+}, Co^{2+}, and Ni^{2+} with hen egg-white lysozyme.[313] The binding constants were determined using the decrease occurring in the negative ellipticity of the c.d. band at 305 nm, which reflects the interaction between tryptophan-108 and glutamic acid-35. On the basis of changes in the c.d. maxima at 305 and 295 nm, it was also shown that the metal ions can interact with lysozyme binding either a substrate (glycol-chitin) or an inhibitor (2-acetamido-2-deoxy-D-glucose, *NN'*-diacetylchitobiose, or *NN'N"*-triacetylchitotriose). The binding constant of manganese(II) ions was increased by a factor of two when the di- or tri-saccharide had been bound to the enzyme, whereas the binding constant for this ion to the complex of the enzyme and 2-acetamido-2-deoxy-D-glucose was only slightly larger than that for the free enzyme. The binding constants of the di- and tri-saccharides to the manganese(II) ion–enzyme complex were also about twice as large as those determined in the absence of the metal ion, whereas the binding constant for 2-acetamido-2-deoxy-D-glucose in the presence of manganese(II) ions was almost the same as that in its absence. In the case of cobalt(II) ions, the binding of sugars was indicated to be independent of that of the metal ion. The activity of lysozyme towards glycol-chitin was explained in terms of kinetic and thermodynamic parameters related to the binding.

C.d. spectroscopy has also been used to study the charge-transfer binding of *N'*-methylnicotinamide chloride to tryptophan-62 of hen egg-white lysozyme and to tryptophan derivatives.[314] Data for the derivatives at various pH values and ionic strengths suggested that electrostatic interactions between the positively charged pyridinium ion and charges located near the indole residue are very important for the charge-transfer interactions. The dependence on pH of the association constant for lysozyme,

[312] T. Monodane, S. Hara, and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 1175.
[313] K. Ikeda and K. Hamaguchi, *J. Biochem.* (*Japan*), 1973, **73**, 307.
[314] K. Ikeda and K. Hamaguchi, *J. Biochem.* (*Japan*), 1973, **74**, 221.

corrected for the electrostatic interaction, indicated that two ionizable groups, having pK values of 5.8 and 4.5, were slightly perturbed to lower values on charge-transfer binding of N'-methylnicotinamide. Assignment of the former to the carboxy-group of glutamic acid-35 suggested that the electronic state of tryptophan-62 is linked with the ionization of this carboxylic acid group, which is spatially separated from tryptophan-62. It was also demonstrated that another ionizable group, with a pK value of 3.5, affects the molar ellipticity of the complex.

Hen egg-white lysozyme has been used in a comparison of protein structure in crystals and in solution by laser-Raman scattering.[315] Good agreement between the Raman spectra of lysozyme chloride crystals (in 100% relative humidity) and of lysozyme in solution (at pH 4.5) in the regions of the two amide bands indicated that the main-chain conformation is the same in the two phases. A number of differences in the spectra were interpreted in terms of changes in the conformations of side-chains. Comparison of the spectra with that for the enzyme in lyophilized form led to the conclusion that lyophilization caused appreciable conformational changes in both the main- and side-chains.

Structure–activity relationships for hen egg-white lysozyme have also been deduced from derivatizations of the enzyme. Modification of the amino-groups by succinylation, guanidination, and maleylation resulted in changes in the aromatic region of the c.d. spectra, and in the activity of the enzyme towards glycol-chitin.[316] It was suggested that the electronic state of the tryptophanyl residues is changed by these modifications. The dependence on pH of shifts in the spectra at alkaline pH could well be interpreted in terms of changes in the mean net charge on the lysozyme molecule. This and other observations, including the fact that the dependence on pH on the acid side was not affected by the mean net charge on the protein molecule, suggested that glutamic acid-35 is located in a very special environment, and that it is shielded from changes occurring in the rest of the molecule. Reduction of the disulphide bonds of the enzyme with 2-mercaptoethanol in urea solution, followed either by treatment with iodoacetic acid and carboxymethylation or by reaction with methyl 4-nitrobenzene-sulphonate and methylation, gave derivatives whose chains, according to o.r.d. and c.d. measurements, were significantly more unfolded than those of the natural enzyme.[317] The stabilities of the derivatives were investigated, and the stabilized structure of carboxymethylated lysozyme had o.r.d. parameters closely resembling those of the native enzyme. The S-methylated enzyme cross-reacted appreciably with antisera to the native enzyme, whereas carboxymethylated lysozyme gave no cross-reaction. The complete loss of enzymic activity by derivatization with either method suggested that more rigid structural requirements are needed for enzymic

[315] N. T. Yu and B. H. Jo, *Arch. Biochem. Biophys.*, 1973, **156**, 469.
[316] Y. Nakae, K. Ikeda, and K. Hamaguchi, *J. Biochem. (Japan)*, 1973, **73**, 1249.
[317] C. L. Lee and M. Z. Atassi, *Biochemistry*, 1973, **12**, 2690.

activity than for immunological cross-reactivity. These findings indicated that it is feasible, at least to a limited extent, to effect a stabilized structure in the *S*-methylated enzyme, owing to the ability of the *S*-methyl groups to participate in non-polar interactions. On the other hand, the directive effect of long-range interactions in carboxymethylated lysozyme is ineffective because the formation of a stable, unfolded structure is prevented by steric effects and by repulsion between anionic carboxymethyl groups as they approach one another. A variety of reactivities of the carboxy-groups of hen egg-white lysozyme was found when the enzyme was coupled (with the aid of a carbodi-imide) to sulphanilic acid as a chromophoric nucleophile.[318] When the reaction was carried out in the presence of an inhibitor of lysozyme, aspartic acid-101 and glutamic acid-35 were found to have reduced reactivities. Labelling of the lysozyme with fluorescein isothiocyanate, to give derivatives containing different proportions of the substituent, did not appreciably alter the activity of the enzyme towards glycol-chitin.[319] No measurable change occurred in the 210—250 nm region of the c.d. spectrum on derivatization, whereas the c.d. spectrum in the aromatic region (250—300 nm) and the tryptophanyl fluorescence spectra changed considerably with the degree of labelling. It was suggested that some interaction between the fluorescein isothiocyanato-groups and amino-acid residues (probably the tryptophanyl residues) occurs at high degrees of labelling.

Using immunologically active peptides from hen egg-white lysozyme, region-specific antibodies to the enzyme have been raised.[320] Relationships between the antibody specificity and immunological neutralization of the enzyme have been studied with the aid of the specific antibodies.[320, 321]

Optical-mixing spectroscopy has been used to measure the translational diffusion coefficient of hen egg-white lysozyme in buffered solutions of guanidine hydrochloride.[322] The values of the coefficient, in conjunction with other hydrodynamic parameters, indicated that the enzyme retains a compact configuration on denaturation. However, the spectral shape of the light scattered by lysozyme did not permit different models of denaturation to be distinguished, in view of the similar diffusion coefficients of the native and the denatured enzyme. Assuming a model in which the enzyme can exist in only two states (native and denatured), the fraction of each present at different concentrations of the denaturant was calculated. Investigation of the thermal denaturation of lysozyme by differential-scanning microcalorimetry has shown that the heat capacity of a solution of the enzyme changes from the very start of heating and increases linearly at first.[323] At

[318] K. J. Kramer and J. A. Rupley, *Arch. Biochem. Biophys.*, 1973, **156**, 414.
[319] M. Hiramatsu, N. Okabe, and K. Tomita, *J. Biochem. (Japan)*, 1973, **73**, 971.
[320] H. Fujio, N. Sakato, and T. Amano, *Biken J.*, 1971, **14**, 395.
[321] N. Sakato, H. Fujio, and T. Amano, *Biken J.*, 1972, **15**, 135.
[322] S. B. Dubin, G. Feher, and G. B. Benedek, *Biochemistry*, 1973, **12**, 714.
[323] N. N. Khechinashvili, P. L. Privalov, and E. I. Tiktopulo, *F.E.B.S. Letters*, 1973, **30**, 57.

higher temperatures, the heat capacity rises rapidly to a sharp peak, and the resulting intensive absorption of heat is apparently connected with the main process of thermal denaturation. The dependences on temperature of the differential spectra and the enthalpy of transition of the lysozyme and of the optical density of a solution of the enzyme at different wavelengths were also investigated. The products of denaturation of dry hen egg-white lysozyme by γ-irradiation have been studied kinetically.[324] The aggregation of heavily damaged products was delayed in the presence of substrates for the enzyme, indicating a possible involvement of the active site in this process. The activation energy for γ-irradiated lysozyme and the apparent affinity constant for irradiated lysozyme and its salt-soluble fraction (F-II) were found to be the same as for the natural enzyme, but V_{max} decreased on irradiation. A heavily damaged, salt-soluble fraction (F-I) and salt-insoluble products were shown to be almost inactive. Difference spectra of glycol–chitin complexes of native and irradiated lysozymes and the F-II fraction showed similarities, but the spectrum of the F-I fraction possessed abnormal characteristics, indicative of alterations of the protein chain in and around a tryptophanyl residue. It was proposed that damaged enzyme molecules suffer further modifications during fractionation, resulting in loss of enzymic activity.

Hen egg-white lysozyme has been used in the enzymic synthesis of oligosaccharides containing 2-acetamido-2-deoxy-D-xylose from *NN'N"N"'*-tetra-acetylchitotetraose and 2-acetamido-2-deoxy-D-[5-^{3}H]xylose.[325] The effects of hen egg-white lysozyme and chitinase on the spherules of *Coccidioides immitis in vitro*,[326] and those of lysozyme on the diffusion of anions and cations from phospholipid liposomes [327] have been investigated.

Two solid-phase (Merrifield) syntheses of hen egg-white lysozyme have been reported.[328] An improved procedure yielded a product of the correct molecular weight (according to gel filtration) and a non-reducible form of high molecular weight. Only the former possessed enzymic activity, which, for the crude preparation, was 0.5—1.0% of that of the native enzyme. Following chromatography on chitin, the specific activity was 2—3% of that of the native form or 9—25% of that of a native form that had been subjected to the same experimental conditions as the synthetic product.

In a study of the reaction of tetranitromethane with turkey egg-white lysozyme, it was found that one tyrosyl residue remained unreactive even at the highest concentration of reagent.[329] The various products possessed appreciable enzymic activity (minimum 40%), and gel filtration revealed that nitration was accompanied by polymerization of the enzyme.

[324] D. J. Marciani and B. M. Tolbert, *Biochim. Biophys. Acta*, 1973, **302**, 376.
[325] P. van Eikeren, W. A. White, and D. M. Chipman, *J. Org. Chem.*, 1973, **38**, 1831.
[326] M. Collins and D. Pappagianis, *Infection and Immunity*, 1973, **7**, 817.
[327] J. H. Kaplan, *Biochim. Biophys. Acta*, 1973, **311**, 1.
[328] J. J. Sharp, A. B. Robinson, and M. D. Kamen, *J. Amer. Chem. Soc.*, 1973, **95**, 6097.
[329] W. L. Riggle, J. A. Long, and C. L. Borders, jun., *Canad. J. Biochem.*, 1973, **51**, 1433.

Tyrosine-23 was found to be most readily nitrated and, at high tetranitromethane : lysozyme ratios, tryptophanyl as well as tyrosyl residues were nitrated.

Lysozyme from the denervated gastrocnemius muscle of frogs has been purified by ion-exchange chromatography and was found to be stable at 100 °C for a short time.[330] The specific activity of the enzyme increased progressively with increasing denervation. The significance of the lysozyme with respect to atrophic processes of the muscle was discussed.

Evidence has been presented for the presence of lysozyme in organs of fresh-water fish, *viz.* three Indian carps [*Catla catla*, *Labeo rohita* (Rohu), and *Cirrhina mirgala* (Mirgal)] and *Tilapia mosambica*, and salt-water fish, *viz. Scatophagus argus* (Scat), *Therapon puta* (Target perch), and *Epinephalus* sp. (Hekru).[331] The catalytic properties of the enzymes from livers of species living in different environments varied considerably with respect to the ionic strength and pH required for maximum activity. Lysozyme from fresh-water fish exhibited one pH optimum (5.4) and required low concentrations of buffer for activity, whereas lysozyme from marine fish exhibited two pH optima (6.2 and 9.2) and required relatively high concentrations of buffer. A procedure for the partial purification of lysozyme from the liver of *T. mosambica* was reported.

Bacteriolytic activity has been shown to develop in the haemolymph of larvae of wax moths (*Galleria mellonella*) and silk worms (*Bombyx mori*) after injection with micro-organisms, but not after injection with saline.[332] On purification by gel filtration and ion-exchange chromatography, the lytic enzymes were found to be relatively small-sized proteins with properties (*e.g.* stability to heat, and optima for pH and ionic strength) similar to those of hen egg-white lysozyme, but with specific activities approximately six times that of the enzyme from egg-white. The enzyme from *G. mellonella* released a reducing sugar, which was identified as *N*-acetylmuramic acid, from bacterial cell walls. These properties of the insect proteins permitted them to be classified as lysozymes.

The autolytic glycosidase of *Bacillus subtilis* has been shown to degrade the cells and cell walls of *Micrococcus lysodeikticus*, whereas the autolytic amidase does not.[333] The criteria used to establish this included determination of the chemical bonds broken, heat-inactivation kinetics, pH–activity dependence (the glycosidase has an optimum pH of 4—5), and physical separation of the two enzymes by ion-exchange chromatography. The autolytic glycosidase can be regarded as a lysozyme.

Incubation of a crude preparation of an extracellular enzyme from *Bacillus subtilis* with *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (A) and *O*-[2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranosyl]-(1 → 4)-2-

[330] R. V. Krishnamoorthy, *Enzymologia*, 1972, **43**, 353.
[331] K. Sankaran and S. Gurnani, *Indian J. Biochem. Biophys.*, 1972, **9**, 162.
[332] R. F. Browning and W. J. Davidson, *Comp. Biochem. Physiol.*, 1973, **45B**, 669.
[333] D. Fan and M. M. Beckman, *J. Bacteriol.*, 1973, **114**, 804.

acetamido-2-deoxy-D-glucose (B) resulted in the release of 2-acetamido-2-deoxy-D-glucose in both cases.[334] It was concluded that the preparation contains *exo*-lysozyme activity. The pH optimum (5.0—5.2) of this activity differs from that (5.9—6.0) of an *exo*-β-acetamidodeoxyglucosidase known to be produced by the organism under the same conditions. Attempts were made to inhibit the lysozymal activity using antibodies to *exo*-β-acetamidodeoxyglucosidase. Although this treatment did not inhibit the lysozymal activity against disaccharide B, the activity against disaccharide A and 4-methylumbelliferyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside was reduced. Since the possibility exists that lysozymal activity is due to an *exo*-β-acetamidodeoxyglucosidase possessing a broader specificity than previously recognized, the effects of inhibitors of β-acetamidodeoxyglucosidase (*e.g.* 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucono-1,5-lactone) on the lysozyme were investigated, but they were found to have no effect. However, the lysozyme was inhibited by muramic acid. It was concluded that the activity is distinct from the *exo*-β-acetamidodeoxyglucosidase and that it is a newly identified *exo*-lysozyme.

Streptomyces griseus and *S. globisporus* have been demonstrated to possess high lytic activities against cariogenic Streptococci.[335] Enzyme production by *S. griseus* accompanied spore formation during surface culture, whereas that of *S. globisporus* occurred in submerged culture. The enzymes possess wide substrate specificities against all groups of cariogenic Streptococci, and the possible prevention of dental caries by the enzymes was investigated.

Active, water-insoluble derivatives of lysozyme have been prepared in aqueous solution by covalent coupling of the enzyme to either 4-diazobenzylcellulose or poly-[*N*-4-diazobenzamidoethyl(acrylamide)],[336] and in non-aqueous media by covalent coupling of the enzyme, with the aid of *NN*-carbonyldi-imidazole, to a copolymer of styrene and 4-vinylbenzoic acid dissolved in DMF.[337] Lysozyme has also been immobilized, with retention of activity, by impregnation of pre-swollen collagen with a solution of the enzyme.[84]

endo-β-1,4-Mannanases

An enzyme that hydrolyses konjac mannan has been extracted from germinating konjac (*Amorphophallus konjac*) tubers; it was purified by chromatography on an ion-exchange resin and alkali-swollen cellulose, followed by gel filtration.[338] The purified enzyme, which possesses a pH

[334] L. A. del Rio, R. C. W. Berkeley, S. J. Brewer, and S. E. Roberts, *F.E.B.S. Letters*, 1973, **37**, 7.
[335] K. Yokogawa, S. Kawata, and Y. Yoshimura, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 2055.
[336] R. Datta, W. Armiger, and D. F. Ollis, *Biotechnol. and Bioeng.*, 1973, **15**, 993.
[337] G. J. Bartling, H. D. Brown, and S. K. Chattopadhyay, *Nature*, 1973, **243**, 342.
[338] N. Sugiyama, H. Shimahara, T. Andoh, and M. Takemoto, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 9.

optimum of 4.7 and a temperature optimum of 40 °C, was stable within the pH range 4—8, but was rapidly inactivated by temperatures above 50 °C. Hydrolysis of konjac mannan proceeded by a typical, random mechanism, and kinetic parameters were determined for the enzyme.

Mannanases (Miscellaneous)

The production of mannanase activity by cultures of a strain of *Bacillus subtilis* has been investigated.[257]

A novel glycanase, which specifically hydrolyses a *Salmonella anatum* polysaccharide consisting of α-galactosylmannosylrhamnosyl repeating units, has been found in association with the phage particles of *Salmonella*.[258] The activity, which was detected by measuring the increase in reducing power of a solution of the substrate, was inhibited by bivalent cations. A β-galactosylmannosylrhamnosyl-type polysaccharide did not serve as a substrate for the enzyme. It is possible that the enzyme is responsible for the specific adsorption of phage particles onto bacteria.

Pectate Lyases

The initial rates of reaction of pectate lyases from an *Arthrobacter* species and *Bacillus polymyxa* on a series of pectins having different degrees of esterification have been determined.[339] Values of V_{max} and K_m^{-1} were found to be maximal for a 44%-esterified pectin, whereas other enzymes showed maxima for these values at lower degrees of esterification. It was concluded that pectate lyases can efficiently degrade most naturally occurring pectins without invoking the action of a pectinesterase.

The effect of nalidixic acid on the formation of pectolytic enzymes by cultures of whole cells of *Erwinia aroideae* has been investigated.[340] An increase in pectolytic activity was indicated when the activity was measured relative to the decrease in viscosity of solutions of the pectin. However, determination of the pectate lyase and polygalacturonase activities by measurements of the optical density at 230 nm and the liberated aldehydic groups, respectively, did not indicate any stimulation. Analysis of the pectolytic enzymes by chromatography on carboxymethylcellulose demonstrated a difference in the enzymes present in normal and nalidixic acid-treated systems.

A study has been made of the pectolytic enzymes produced by *Pseudomonas fluorescens*, an organism causing 'pink eye' disease of potato tubers.[341] Pectate lyase activity was observed in, and extracted from, potato tubers inoculated with the organism. Ion-exchange chromatography of the extract gave two fractions containing pectate lyase, one of which possessed greater activity than the other. The two lyases, which were

[339] F. M. Rombouts and W. Pilnik, *J. Microb. Serol.*, 1972, **38**, 627.
[340] S. Kamimiya, K. Izaki, and H. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2367.
[341] S. S. Hagar and G. A. McIntyre, *Canad. J. Botany*, 1972, **50**, 2479.

homogeneous on electrophoresis on cellulose acetate, exhibited maximum activity at pH 8.5—9.5 and showed a preference for polygalacturonate, rather than pectin, as a substrate. The activities of both enzymes were enhanced by the addition of calcium ions, whereas they were completely inhibited by H_4edta. T.l.c. of the products of hydrolysis of polygalacturonate revealed the presence of unsaturated oligosaccharides and pectic fragments, but the latter were not present after lengthy incubations. No saturated oligosaccharides were obtained from the digests, indicating the absence of polygalacturonases. Molecular weights of 1.80×10^4 and 2.25×10^4 for the more- and less-active species, respectively, were estimated by gel filtration.

The production of pectate lyase, polygalacturonase, *exo*-polygalacturonase, and cellulase by virulent and avirulent isolates of *Sclerotium bataticola* during development of the disease in sunflower (*Helianthus annuus*) plants has been investigated.[244] The pectate lyase and the cellulase activities increased with time after inoculation. Although the two enzymes are considered not to be important in the penetration of *S. bataticola* into sunflower plants, they are involved in the development of the pathogen. The ability of the organism to produce these enzymes in plants was shown to be closely related to the virulence of the respective isolates.

Pectin Lyases

Purified pectin lyase from *Aspergillus japonicus* showed almost the same clarifying activity as the crude enzyme, but polygalacturonase did not effect the clarification of apple juice.[342] Both enzymes showed clarifying activity with grape juice. Variations in the susceptibilities from juice to juice were found to be related principally to the degree of esterification of pectins in the juice. A mixture of pectin lyase and polygalacturonase brought about clarification of grape juice more rapidly than did either enzymes alone, indicative of a synergistic effect.

The use of pectin lyase from *Aspergillus sojae* for the clarification of fruit juices is considered to have advantages over the use of either polygalacturonase or a mixture of polygalacturonase and pectinesterase, since it produces neither methanol nor carboxy-groups.[343]

The formation of pectolytic enzymes by whole cells of *Erwinia aroideae* was markedly stimulated when nalidixic acid was added to the culture medium.[340] This stimulation was recognized by a decrease in the viscosity of a solution of pectin. However, measurements of the pectate lyase and polygalacturonase activities by determination of optical density at 230 nm and the liberated aldehydic groups, respectively, did not indicate any stimulation. Analysis of the pectolytic enzymes by chromatography on carboxymethylcellulose demonstrated a difference in the enzymes present in normal and nalidixic-acid-treated systems.

[342] S. Ishii and T. Yokotsuka, *J. Agric. Food Chem.*, 1973, **21**, 269.
[343] S. Ishii and T. Yokotsuka, *J. Agric. Food Chem.*, 1972, **20**, 787.

Polygalacturonases

A study of the polygalacturonase activities of the surface snails *Eulota maackii* and *Helix pomatia* showed that the polygalacturonase activity of the former reaches a maximum at certain times of the year, and that the enzyme exhibits maximum activity against zosterine at pH 4.0—4.4 and 45 °C.[164] Zosterine-hydrolysing preparations from both species were purified by gel filtration and were found to possess *endo* activity, digesting zosterine and galacturonan to fragments of high and low molecular weights. The products of low molecular weight were more acidic than zosterine itself, whereas the converse held for the products of high molecular weight. Among the products of low molecular weight were oligosaccharides of D-galacturonic acid, although D-galacturonic acid was the major component. Thus, the zosterinase activities were concluded to be a complex of polygalacturonases and *exo*-polygalacturonases.

Low polygalacturonase activity was found in tissues of the orange tree (*Citrus sinensis*), and the activity remained unaltered one day after treatment of the trees with cycloheximide.[235]

Two polygalacturonate-hydrolysing enzymes have been separated from extracts of ripe peaches (*Prunus persica*) by gel filtration.[344] One of the enzymes (pH optimum 4) was found to cleave randomly the molecular chain of poly(D-galacturonic acid); it was most reactive with substrates of medium molecular weight and catalysed the release of water-soluble, 70% ethanol-insoluble pectin from washed cell walls of peaches. The other enzyme possesses the characteristics of an *exo*-polygalacturonase.

Two polygalacturonases (I and II) have been separated from extracts of ripe tomato fruits by chromatography on DEAE-Sephadex; polygalacturonase II was found to be the predominant species in all materials examined.[345] The enzymes (molecular weights: I, 8.4×10^4 and II, 4.4×10^4) differed in their stabilities to temperature and pH. The pHs of optimum activity of both enzymes were shifted to the acid side with decreasing size of the substrate and on addition of sodium chloride to the digests, but polygalacturonase I was less affected than polygalacturonase II. The latter enzyme was much more effective than polygalacturonase I in reducing the viscosity of solutions of pectic acid.

Purification to ultracentrifugal homogeneity of a polygalacturonase from culture fluids of a strain of *Acrocylindrium* species has been reported.[346] Whereas the hydrolysis of α-(1 → 4)-linked di- and tri-saccharides of D-galacturonic acid by the enzyme was undetectable, the tetrasaccharide was hydrolysed to the trisaccharide and D-galacturonic acid. The corresponding pentasaccharide was hydrolysed either to the tetrasaccharide and D-galacturonic acid or to the di- and tri-saccharides, but the rate of formation of the latter products was four times greater. It was concluded

[344] R. Pressey and J. K. Avants, *Plant Physiol.*, 1973, **52**, 252.
[345] R. Pressey and J. K. Avants, *Biochim. Biophys. Acta*, 1973, **309**, 363.
[346] H. Kimura, F. Uchino, and S. Mizushima, *J. Gen. Microbiol.*, 1973, **74**, 127.

that the enzyme can hydrolyse D-galacturonosidic bonds at either the reducing end or the third unit from the non-reducing end of a poly-(D-galacturonic acid). Attention was drawn to the different ways in which this and other polygalacturonases hydrolyse poly(D-galacturonic acid).

Purification of a polygalacturonase from *Aspergillus japonicus* by ion-exchange chromatography and gel filtration yielded the enzyme (molecular weight 3.55×10^3) in a form that was homogeneous on examination by ultracentrifugation and disc electrophoresis.[347] The enzyme (pH optimum 4.5) rapidly reduced the viscosity of a solution of pectic acid, releasing reducing groups in a random manner with the formation of D-galacturonic acid and tri- and di-saccharides thereof. The purified enzyme was able to macerate various kinds of plant tissues unaided.

Polygalacturonase from *Aspergillus japonicus* effected the clarification of grape juice but not apple juice, whereas purified pectin lyase from *Aspergillus japonicus* clarified both juices.[342] Variations in the susceptibilities of the juices to the polygalacturonase were found to be related principally to the degree of esterification of the pectins. A mixture of the poly-galacturonase and pectin lyase brought about clarification of grape juice more rapidly than did either enzyme alone, indicative of a synergistic effect.

The effects of vegetable extracts on the activity of polygalacturonase from *Aspergillus niger* have been studied.[348] Extracts of white radish (*Raphanus sativum*), green pepper (*Capsicum frutescens*), and leek had no effect, but the activity of the enzyme was markedly inhibited by extracts of garlic (*Allium sativum*) and onion (*Allium cepa*).

The formation of pectolytic enzymes by whole cells of *Erwinia aroideae* was greatly stimulated when nalidixic acid was added to the culture medium.[340] This stimulation of activity was indicated by a decrease in the viscosity of a solution of pectin. However, measurement of the poly-galacturonase and pectate lyase activities by determinations of liberated aldehydic groups and the optical density at 230 nm, respectively, did not indicate any stimulation in the system. Analysis of the pectolytic enzymes by chromatography on carboxymethylcellulose demonstrated a difference in the enzymes present in normal and nalidixic-acid-treated systems.

Polygalacturonase and other pectolytic enzymes have been shown to be produced by virulent and avirulent isolates of *Sclerotium bataticola* during development of the disease in sunflower (*Helianthus annuus*) plants.[244] Both polygalacturonase and *exo*-polygalacturonase were found to be important in the penetration of sunflower plants by the organism and in the early stages of pathogenesis. The virulence of the isolates was found to be related to the ability of the organism to produce these enzymes in the host.

The polygalacturonate-hydrolysing activity of culture fluids from *Verticillium albo-atrum* has been purified by gel-permeation and ion-

[347] S. Ishii and T. Yokotsuka, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1885.
[348] H. A. Al-Jasim and M. M. Barakat, *J. Sci. Food Agric.*, 1972, **24**, 119.

exchange chromatography and by double isoelectric focusing.[349] These procedures yielded two active fractions, one of which possessed the characteristics of an *exo*-polygalacturonase. The other fraction, which was homogeneous on polyacrylamide-gel electrophoresis, released oligosaccharides of D-galacturonic acid from sodium polypectate and pectin, although the polypectate is the more effective substrate. D-Galacturonic acid was not released by the enzyme from either substrate until incubation had been maintained for a long period. The enzyme is considered to be a polygalacturonase. The enzyme (molecular weight 3.45×10^4, p*I* 9.7) possessed different pH optima (5.0 and 6.0, respectively) for pectin and sodium polypectate, and was very effective in macerating plant tissues. The polygalacturonase was found to be exceptionally stable when stored in solution at 4 °C, and the activity and pH optima were unaffected by calcium and magnesium ions, H_4edta, or disodium oxalate.

Polygalacturonase has been allowed to react with titanium chelates of poly-(4- and 5-acrylamidosalicylic acids), but inactivation of the enzyme was attributed to inhibition by the matrices.[223]

exo-Polygalacturonases

A study of the enzymic activities of the surface snails *Eulota maackii* and *Helix pomatia* has shown that the zosterinase activity of the former reached a maximum at certain times of the year, and that the enzyme exhibited maximum activity towards zosterine at pH 4.0—4.4 and 45 °C.[164] Zosterine-hydrolysing preparations from both species were purified by gel filtration, and were able to degrade zosterine and galacturonan into fragments of high and low molecular weights. The products of low molecular weight from zosterine were more acidic than zosterine itself, whereas the converse held for the products of high molecular weight. Among the products of low molecular weight were oligosaccharides of D-galacturonic acid, although D-galacturonic acid was the major component of this fraction. The zosterinase activities were concluded to be a complex of *exo*-polygalacturonases and polygalacturonases.

Two polygalacturonate-hydrolysing enzymes have been separated from extracts of ripe peaches (*Prunus persica*) by gel filtration.[344] One of the enzymes was found to hydrolyse poly(D-galacturonic acid) from the non-reducing terminal with the release of D-galacturonic acid. The enzyme functioned optimally at pH 5.5, required calcium(II) ions for activity, and hydrolysed substrates of low molecular weight most readily. The enzyme was classified as an *exo*-polygalacturonase, whereas the other enzyme exhibited the characteristics of a polygalacturonase.

exo-Polygalacturonase and other pectolytic enzymes have been shown to be produced by virulent and avirulent isolates of *Sclerotium bataticola*

[349] H. W. Mussell and B. Strouse, *Canad. J. Biochem.*, 1972, **50**, 625; see also erratum, *Canad. J. Biochem.*, 1972, **50**, 1416.

during development of the disease in sunflower (*Helianthus annuus*) plants.[244] The *exo*-polygalacturonase and polygalacturonase were found to be important in the penetration of sunflower plants by the organism and in the early stages of pathogenesis. The virulence of the isolates is related to the ability of the organism to produce these enzymes in the host.

The polygalacturonate-hydrolysing activity of culture media of *Verticillium albo-atrum* has been purified by gel-permeation and ion-exchange chromatography and double isoelectric focusing.[349] These procedures yielded two active fractions, one of which possessed the characteristics of a polygalacturonase. The other fraction, which was homogeneous on polyacrylamide-gel electrophoresis, released D-galacturonic acid from sodium polypectate and pectin, although the latter is the better substrate. The fraction exhibited no pectate lyase activity and is considered to contain an *exo*-polygalacturonase. The enzyme (molecular weight 4.4×10^4, p*I* 6.5) possessed different pH optima (4.5 and 5.3, respectively) for the hydrolyses of pectin and sodium polypectate, but was not as effective as the polygalacturonase in macerating plant tissues. The activity and pH optima of the *exo*-polygalacturonase were unaffected by calcium and magnesium ions, H_4edta, or disodium oxalate. The *exo*-polygalacturonase was exceptionally stable when stored in solution at 4 °C.

Pullulanases

The specific activity of pullulanase from an *Aerobacter* species has been determined in terms of the cleavage of glycosidic bonds per minute per milligram of enzyme protein at a substrate concentration of 2%, and the values so obtained were compared with those for isoamylase from a *Pseudomonas* species.[285] Values of 3—5 and 110—280 for amylopectins, 0.5—1.2 and 110—280 for glycogens, and 53 and 1.1 μmol for pullulan, respectively, were obtained for the two enzymes. K_m Values for the action of the pullulanase and isoamylase were found to be $6—10 \times 10^{-3}$ for amylopectins, $2—5 \times 10^{-2}$ and $1—2 \times 10^{-4}$ for glycogens, and 1.7×10^{-5} and 2×10^{-3} g ml^{-1} for pullulan, respectively.

Extracellular pullulanase has been purified from culture fluids of *Aerobacter aerogenes* by ion-exchange chromatography and gel filtration.[350] The enzyme was obtained in the form of fine, colourless rods by addition of a saturated solution of ammonium sulphate to the purified enzyme in solution. On ultracentrifugation, the enzyme gave a single, sharp, and symmetrical Schlieren peak ($S_{20,w} = 4.39\,S$). Polyacrylamide-gel electrophoresis at pH 8.4 gave a major band and two sub-bands; the molecular weight of the principal enzyme was estimated to be 5.8×10^4 by sedimentation equilibrium and 6.6×10^4 by polyacrylamide-gel electrophoresis. The optimum pH and temperature for enzymic action are pH 6.5 and 50 °C, respectively.

[350] S. Ueda and R. Ohba, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 2381.

Rhamnanases

A novel glycanase, which specifically hydrolyses a *Salmonella anatum* polysaccharide consisting of α-galactosylmannosylrhamnosyl repeating units, was found to be associated with the *Salmonella* phage.[258] The enzyme, which was detected by an increase in the reducing power of a solution of the substrate, was inhibited by bivalent cations and was inactive against a β-galactosylmannosylrhamnosyl-type polysaccharide. The enzyme is considered to be responsible for the specific adsorption of phage particles on to bacteria.

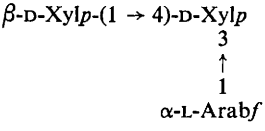
Trehalases

The trehalase activities of the small intestines of humans and non-human primates (Lorisidae, Hapalidae, and Cebidae families) have been determined, and the slow loris (*Nycticebus coucang*) was found to possess the highest trehalase activity.[73]

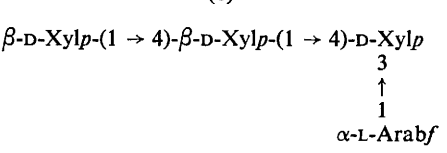
Studies of various glycosidase and trehalase activities in the small intestines of cats, rabbits, guinea pigs, mice, and rats showed that each enzyme exhibits maximal activity in the jejunal segments of all the species, and that cats have a low trehalase activity.[99] The effects of feeding and starving the animals on the enzymic activities were investigated.

Xylanases (Miscellaneous)

A xylanase from *Aspergillus niger* has been applied to an arabinoxylan from rice straw.[351] Structural identification of the products of enzymolysis showed that a trisaccharide (6) and a tetrasaccharide (7) had been released.



(6)



(7)

The production of xylanase activity by cultures of a strain of *Bacillus subtilis* has been investigated.[257]

Two xylanases have been separated from a cellulosic complex of a species of Basidiomycete by ion-exchange chromatography on DEAE-Sephadex.[139] The characteristics of the enzymes were investigated and, during the course of the work, four other polysaccharidases were found.

[351] S. Takenishi and Y. Tsujisaka, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1385.

Carbohydrate Epimerases

The interconversion of sugars in biological systems by the action of epimerases on sugar nucleotides has been reviewed,[352] and some recent advances in the field of epimerase-catalysed transformations of sugars involving pyridine nucleotides have been discussed.[353]

Carbohydrate Isomerases

L-Arabinose Isomerases.—A *Streptomyces* species isolated from sea water has been found to produce L-arabinose isomerase when grown on L-arabinose.[354] The enzyme was purified by treatment with manganese chloride, fractionation on poly(ethylene glycol), ion-exchange chromatography, and gel filtration. In its purified form the enzyme was shown to be specific for L-arabinose, and it was inhibited competitively by L-arabinitol, ribitol, and xylitol. After dialysis against H_4edta, the enzyme could be re-activated by manganese(II) or cobalt(II) ions.

Glucose Isomerases.—Crystalline glucose isomerase from *Bacillus coagulans* has been demonstrated to consist of subunits by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate.[355] The enzyme dissociated into subunits on pre-incubation with the detergent in the presence of either manganese or cobalt chlorides, but not in the absence of the salts. In the presence of urea, however, dissociation into subunits did not occur and the activity could be completely recovered by dilution. Manganese and cobalt chlorides did not affect the activity in the presence of urea.

The properties of the glucose isomerase from *Streptomyces griseolus* and the optimum conditions for activity and stability of the enzyme have been investigated.[356] The characteristics of the free enzyme were compared with those of an immobilized form obtained by physical entrapment of the enzyme in filamentous structures.

Collagen has also been used as a carrier for an active, immobilized form of glucose isomerase.[84] Immobilization was accomplished by electrodeposition from a collagen dispersion containing the dissolved enzyme; the collagen–enzyme complex was obtained in membranous form. Heat-treated cells of *Streptomyces phaeochromogenes* containing active glucose isomerase have been immobilized by binding to collagen, which was then tanned to the desired mechanical strength.[357] This procedure provided an active, water-insoluble form of the enzyme.

[352] L. Glaser in 'Carbohydrates', ed. G. O. Aspinall, MTP International Review of Science, Organic Chemistry Series One, Butterworths, London, 1973, Vol. 7, p. 191.
[353] M. Akhtar and D. C. Wilton, *Ann. Reports (B)*, 1970, **67**, 572.
[354] K. Yamanaka and K. Izumori, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 521.
[355] G. Danno, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1849.
[356] S. Giovenco, F. Morisi, and P. Pansolli, *F.E.B.S. Letters*, 1973, **36**, 57.
[357] W. R. Vieth, S. S. Wang, and R. Saini, *Biotechnol. and Bioeng.*, 1973, **15**, 565.

Carbohydrate Oxidases

Galactose Oxidases.—The e.s.r. spectrum of copper(II)–galactose oxidase has indicated that the metal is in a pseudo-square-planar environment.[358] The spectrum was unaltered under conditions causing inhibition of the enzyme by superoxide dismutase. No combination of substrates (*e.g.* D-galactose and oxygen) and oxidant traps (superoxide dismutase and catalase) resulted in a reduction of the resonance of copper(II) ions. Thus, the copper(I) enzyme does not appear to be a stable intermediate in the reactions of the enzyme.

Glucose Oxidases.—The determination of D-glucose by a glucose oxidase kit (Glucostat Special) was found to give spurious results when used in the presence of cellobiose.[127] This effect, which is due to a contaminant β-glucosidase, can be overcome by a number of methods, including a dilution technique and inactivation of the β-glucosidase by heat.

A positional isotope effect has been observed in the oxidation of D-glucose by glucose oxidase.[359] Measurements of the reaction rates and of the specific activity of the unreacted sugar showed that D-glucose was oxidized more rapidly than D-[1-^{14}C]glucose, whereas D-[6-^{14}C]glucose was oxidized at the same rate as the unlabelled sugar.

The thermal stability of a glucose oxidase of fungal origin in aqueous solution has been studied as a function of time and temperature.[360] As expected, the rate of inactivation increased with temperature, but measurements on solutions of the enzyme containing water-soluble, synthetic polymers showed that a number of polymers significantly enhanced its thermal stability. Copolymers of vinyl acetate with either vinylpyrrolidone or vinyl alcohol were found to be particularly effective. The molecular weight, concentration, and composition of the added polymer were indicated to be important in the stabilization process.

The reaction catalysed by glucose oxidase from *Aspergillus niger* was markedly inhibited by silver(I), mercury(II), and copper(II) ions, 4-chloromercuribenzoate, and mercuric sulphadiazine, whereas the antimicrobials silver sulphadiazine and silver uracil had no effect.[361] Characteristic difference spectra, due to absorption by the flavine adenine dinucleotide moiety of the enzyme, were obtained on addition of silver(I), mercury(II), and copper(II) ions, and mercuric sulphadiazine, whereas no spectral changes could be observed when silver sulphadiazine and various silver uracil compounds were added. From studies of the overall kinetics, it was apparent that mercuric sulphadiazine inhibited reduction of the flavine adenine dinucleotide moiety, thereby competing with the molecular oxygen

[358] D. J. Kosman, R. D. Bereman, M. J. Ettinger, and R. S. Giordano, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 856.
[359] L. M. Marshall and A. Appiah, *J. Chromatog.*, 1972, **73**, 257.
[360] J. J. O'Malley and R. W. Ulmer, *Biotechnol. and Bioeng.*, 1973, **15**, 917.
[361] M. S. Wysor and R. E. Zollinhofer, *Enzyme*, 1972/3, **14**, 185.

used as a hydrogen-acceptor. The apoenzyme from the glucose oxidase of *A. niger* has been prepared in electrophoretically homogeneous form.[362] Evidence for an interaction between the isoalloxazine moiety of flavine adenine dinucleotide and the apoenzyme was obtained from fluorometric studies on apo- and holo-forms of the enzyme, which had been modified in their tryptophanyl residues by reaction with 2-hydroxy-5-nitrobenzyl bromide and in their tyrosyl residues by reaction with *N*-acetylimidazole.

The distribution and degradation of glucose oxidase from *Penicillium amagasakiense* have been investigated after intravenous injection into mice and rats.[363]

Active, water-insoluble derivatives of glucose oxidase have been prepared by reaction of the enzyme with titanium chelates of alginic acid, chitin, and Celite,[364] with poly(vinyl 4-aminophenoxyacetate),[365] and with glass that had been silanized with 3-aminopropyltriethoxysilane and then treated with thiophosgene.[366] Collagen has also been used as a carrier for immobilized glucose oxidase; the immobilized enzyme was obtained in membranous form following electrodeposition from a dispersion of collagen containing the dissolved enzyme.[84]

Hexose Oxidases.—Hexose oxidase from the red alga *Chondrus crispus* has been purified to homogeneity by means of ion-exchange chromatography, pepsin–trypsin treatment, and gel filtration.[367] The enzyme possesses a molecular weight of 1.3×10^5 (gel filtration), and contains 12 gram atoms of copper per mole and 70% carbohydrate, consisting mainly of galactose and xylose. It oxidized D-galactose, D-glucose, cellobiose, and lactose, the products of oxidation being hydrogen peroxide and the terminal hexonolactones. The enzyme exhibited a pH optimum of 6.3 and a temperature optimum of 25 °C, and was inhibited by diethyldithiocarbamate and, to lesser extents, by hydroxylamine, and cyanide, azide, and acetate ions. The red alga *Euthora cristata* also appears to contain this enzyme.

Proteinases

Bromelains.—Stem bromelain has been isolated from an acetone-produced extract of pineapples by single passage through an affinity column of Sepharose to which 5-aminocaproyl-D-tryptophan methyl ester had been covalently attached.[368] In the presence of organomercury compounds and sodium dodecyl sulphate, gel electrophoresis of the enzyme revealed the presence of a single molecular species (molecular weight 2.3×10^4),

[362] H. Tsuge and H. Mitsuda, *J. Biochem.* (*Japan*), 1973, **73**, 199.
[363] I. Miwa, Y. Tanabe, and J. Okuda, *Chem. and Pharm. Bull.* (*Japan*), 1972, **20**, 2407.
[364] J. F. Kennedy and C. E. Doyle, *Carbohydrate Res.*, 1973, **28**, 89.
[365] H. F. Hixson, *Biotechnol. and Bioeng.*, 1973, **15**, 1011.
[366] B. J. Rovito and J. R. Kittrell, *Biotechnol. and Bioeng.*, 1973, **15**, 143.
[367] J. D. Sullivan and M. Ikawa, *Biochim. Biophys. Acta*, 1973, **309**, 11.
[368] D. Bobb, *Prep. Biochem.*, 1972, **2**, 347.

but reduction of the enzyme in mercaptoethanol converted it into two components (molecular weights 1.5×10^4 and 8.5×10^3). The mechanism of inhibition of the enzymic activity of pineapple-stem bromelain by its specific antibody from rabbit antisera has been studied.[369] The caseinolytic activity was almost completely inhibited by the antibody, and the maximum number of antibody molecules attached to one molecule of antigen was calculated to be five; the antibody : enzyme ratio at which caseinolytic activity disappeared was 2.7—3.0 : 1. However, when substrates of low molecular weight, such as α-*N*-benzoyl-L-arginine ethyl ester, were used, the residual activity of the antibody–enzyme complex was much greater and complete inhibition could not be obtained. The immunological reaction was also studied using chemically modified forms of the bromelain. The data suggested that inhibition of the enzyme by its antibody does not involve direct combination of the antibody with the active site of the enzyme, but rather that it is due to two types of steric hindrance related to the molecular weight of the substrate.

Thrombins.—The purification of bovine thrombin by affinity chromatography on an agarose derivative, to which various benzamidines had been covalently attached, has been investigated.[370] The constants (K_I) for inhibition of the enzyme by 4-aminophenylguanidine, 4-aminobenzamidine, and 3-aminobenzamidine were determined; only those benzamidines with low K_I values and which coupled in high concentration to the agarose derivative were found to be satisfactory for the purification of thrombin. The enzyme was eluted from the column by 3-aminobenzamidine and had retained 90% of the original activity.

Ribonucleases and Deoxyribonucleases

Ribonucleases.—The structure of the carbohydrate moiety of ribonuclease I from bovine pancreas has been reviewed.[204]

Deoxyribonucleases.—Purified deoxyribuclease I (from bovine pancreas) has been proteolysed with pronase to yield a mixture of glycopeptides, which was separated by gel filtration.[371] A number of the glycopeptides contained D-galactose and sialic acid, in addition to D-mannose and 2-acetamido-2-deoxy-D-glucose, but most of the glycopeptides contained only the latter two sugars. Sequence studies were carried out using α-mannosidase and β-acetamidodeoxyglucosidase, and it was concluded that the carbohydrate moiety attached to asparagine-18 of deoxyribonuclease I contains two residues of 2-acetamido-2-deoxy-β-D-glucose proximal to the peptide chain, followed by a variable number (4—10) of α-D-mannose residues. The carbohydrate moiety appears to be similar in structure to that of ribonuclease II.

[369] M. Sasaki, S. Iida, and T. Murachi, *J. Biochem.* (*Japan*), 1973, **73**, 367.
[370] H. F. Hixson and A. H. Nishikawa, *Arch. Biochem. Biophys.*, 1973, **154**, 501.
[371] B. J. Catley, *Arch. Biochem. Biophys.*, 1973, **159**, 214.

Miscellaneous Enzymes

Acetylcholinesterases.—The subunit composition of acetylcholinesterase, a glycoproteinaceous enzyme from the electric eel *Electrophorus electricus*, has been examined.[372] Polyacrylamide-gel electrophoresis of each of the A, C, D, and G forms of the enzyme in the presence of sodium dodecyl sulphate gave rise to two major subunits (molecular weights 9.1×10^4 and 6.2×10^4). Subunits of higher molecular weight were also present and, in particular, a subunit of molecular weight greater than 1.8×10^5 was detected in all but the G form. All the major subunits were found to contain carbohydrate, which appeared to include sialic acid. Molecular weights of 8.4×10^4 for forms A and D, and 7.4×10^4 for forms C and G, were obtained when the larger subunits were examined in more detail in 5% sodium dodecyl sulphate.

***N*-Acetyl-lactosamine Synthetases.**—Affinity chromatography matrices, prepared by reaction of α-lactalbumin with Sepharose cyclic imidocarbonate or by reaction (with the aid of a carbodi-imide) of 1-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-aminohexan-1-ol with a succinylaminoethyl derivative of Sepharose, have been used in the purification of *N*-acetyl-lactosamine synthetase from a mouse mastocytoma.[373] The α-lactalbumin derivative was found to be a better affinant for the enzyme. The properties of the purified enzyme, which catalyses the transfer of D-galactose from UDP-D-galactose to 2-acetamido-2-deoxy-D-glucose, were investigated.

***N*-Acetylneuraminate Lyases.**—An *N*-acetylneuraminate lyase activity, which cleaves *N*-acetylneuraminic acid into 2-acetamido-2-deoxy-D-mannose and (presumably) pyruvate, has been detected in *Corynebacterium diphtheriae* and closely related neuraminidase-producing Corynebacteria.[172] A survey of representative Corynebacteria, plant pathogenic Corynebacteria, Mycobacteria, and Nocardias revealed that only *C. diphtheriae* and closely related species possess both *N*-acetylneuraminate lyase and neuraminidase activities. It was shown that these enzymes can synthesize *N*-acetylneuraminic acid *in vitro*.

Adenylate Cyclases.—A soluble adenylate cyclase of *Brevibacterium liquefaciens* has been purified by ion-exchange chromatography and was found to stain positively with the periodate–Schiff reagent.[374] The enzyme was reversibly stimulated by DL-lactate and by pyruvate, but activation by these metabolites was abolished on incubation with neuraminidase, phospholipase C, or a phospholipase A. Neuraminidase and phospholipase C were equally effective in destroying the response of adenylate cyclase to pyruvate and lactate, whereas phospholipase A affected the response to lactate more than that to pyruvate. These and other results suggested that

[372] J. T. Powell, S. Bon, F. Rieger, and J. Massoulié, *F.E.B.S. Letters*, 1973, **36**, 17.
[373] T. Helting and B. Erbing, *Biochim. Biophys. Acta*, 1973, **293**, 94.
[374] M. H. Chiang and W. Y. Cheung, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 187.

adenylate cyclase activity is associated with a lipoglycoprotein and that the enzyme may have been detached from the bacterial cell membrane.

4-L-Aspartylglycosylamine Amidohydrolases.—The substrate specificities of aspartylglucosylaminase (obtained from porcine serum and kidney) have been studied using 4-L-aspartylaminoglycopyranosylamines of 2-acetamido-2-deoxy-β-D-glucopyranose, 2-acetamido-2-deoxy-β-D-galactopyranose, β-D-galactopyranose, β-D-glucopyranose, and β-D-mannopyranose.[375] Other substrates tested included L-asparagine and L-aspartyl derivatives of cyclohexylamine, aniline, hydroxylamine, and hydrazine. With the exception of aspartyl-cyclohexylamine and -aniline, which served as competitive inhibitors, the compounds tested were degraded at various rates by the aspartylglucosylaminase. The enzymes obtained from serum and kidney differed in their relative rates of reaction and K_m for each substrate. The formation of an intermediate aspartyl–enzyme complex was demonstrated by carrying out the enzymic reaction in the presence of hydroxylamine.

Ceruloplasmins.—A spectroscopic study has been made of the kinetics of aerobic oxidation at 25 °C and pH 5.5 of L-ascorbic-acid-reduced ceruloplasmin from humans.[376] The effects of the concentrations of oxygen and of azide ion on the rates were determined. Time-course studies of the changes in absorbance at 610 and 420 nm showed them to be biphasic, and the effects of azide ion on both phases were investigated. Human ceruloplasmin that had been tritiated at the ε-amino-group of the lysyl residues, by reductive methylation with formaldehyde and sodium borotritide, was shown to retain its oxidase activity.[377] Molecular-sieve chromatography of the tritium-labelled glycoprotein showed that neither its molecular size nor its ability to disappear promptly from the circulatory system after desialylation was altered by methylation. The tritiated enzyme exhibited a half-life in plasma similar to that of the same enzyme labelled in other ways. The reaction of nitric oxide with an oxidized ceruloplasmin from an enriched Cohn fraction has been studied.[378] Nitric oxide formed a diamagnetic charge-transfer complex with type-1, but not with type-2, copper atoms of the enzyme. From a comparison of the intensities of the e.s.r. signals, both before and after treatment with nitric oxide, it was concluded that ceruloplasmin contains one type-2 and either two or three type-1 copper atoms per molecule, depending on the total number of copper atoms present.

Factors, including the injection of adrenocorticotrophic hormone, adrenaline, noradrenaline, or reserpine, affecting the activity of plasma ceruloplasmin in chickens (*Gallus domesticus*) have been investigated.[379]

[375] M. Tanaka, M. Kohno, and I. Yamashina, *J. Biochem.* (*Japan*), 1973, **73**, 1285.
[376] T. Manabe, H. Hatano, and K. Hiromi, *J. Biochem.* (*Japan*), 1973, **73**, 1169.
[377] G. Gregoriadis and B. E. Ryman, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 1134.
[378] R. Wever, F. X. R. Van Leeuwen, and B. F. van Gelder, *Biochim. Biophys. Acta*, 1973, **302**, 236.
[379] B. M. Freeman, A. C. C. Manning, and D. S. Pole, *Comp. Biochem. Physiol.*, 1973, **45A**, 689.

α-Lactalbumins.—Investigations of the molecular conformations and fluorescence properties of human, bovine, goat, and guinea-pig α-lactalbumins have provided support for the validity of the 'lysozyme analogy model' of α-lactalbumin.[380]

The effects of reaction with maleic anhydride on the conformation and function of bovine α-lactalbumin showed that all thirteen amino-groups are accessible to the reagent.[381] Maleylation of these groups produced a highly acidic and expanded molecule; despite differences in physical properties, the derivatized α-lactalbumin was as effective as the original macromolecule in the lactose synthetase reaction, whereas treatment with 2,4,6-trinitrobenzenesulphonic acid caused inactivation.

α-Lactalbumin has been found in the sera of lactating rats, but could not be detected in the sera of either female virgin or male rats.[382] The galactosyltransferase involved in the biosynthesis of lactose and glycoprotein was found in sera of both male and female rats. The results obtained from these and other studies support a model proposed for the secretion of lactose and α-lactalbumin into milk and serum by way of the Golgi apparatus.

An immobilized form of α-lactalbumin has been prepared by covalent coupling to a derivative of Sepharose.[383] The derivative was used as an affinity matrix for a study of complex formation between α-lactalbumin and the A-protein of lactose synthetase. A related, Sepharose-based, immobilized derivative of α-lactalbumin was used for the purification of *N*-acetyl-lactosamine synthetase by affinity chromatography.[372]

Lactose Synthetases.—Affinity chromatography of the purified A-protein of lactose synthetase from human milk on columns of α-lactalbumin (the B-species) covalently attached to Sepharose has been used to study complex formation between the two macromolecules.[383] Differences between the elution volumes of the A-species in the presence of D-glucose or 2-acetamido-2-deoxy-D-glucose increased with increasing concentration of the added monosaccharide. Dissociation constants for the monosaccharides in complex formation were found to be independent of the loading of α-lactalbumin on the column gel and to be similar to the K_m values for the same monosaccharides in the transferase reaction in the absence of α-lactalbumin. This suggested that monosaccharides acting as acceptors of D-galactose in the transferase reaction occupy the same binding site on the A-protein as do monosaccharides involved in complex formation between this species and α-lactalbumin. It was also suggested that interaction between the two species may not directly involve the monosaccharides, but occurs following a conformational change in the A-species induced by the binding of monosaccharides.

[380] P. B. Sommers, M. J. Kronman, and K. Brew, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 98.
[381] B. Kitchen and T. E. Barman, *Biochim. Biophys. Acta*, 1973, **298**, 861.
[382] K. E. Ebner and L. M. McKenzie, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1624.
[383] B. J. Kitchen and P. Andrews, *Proc. Austral. Biochem. Soc.*, 1973, **6**, 18.

Levansucrases.—Two inducible enzymes, namely a levansucrase and a β-fructofuranosidase, have been shown to be responsible for the saccharolytic activity of a strain of *Bacillus subtilis*.[76] Up to 90% of the saccharolytic activity is extracellular, and is due to levansucrase. The levansucrase ($S_{20,w}$ = 4.16 *S*; molecular weight 4.0 × 10^4) was obtained in a pure form by ion-exchange chromatography, and catalysed trans-fructosylation reactions with water, D-glucose, sucrose, and levans as acceptors. The apparent K_m values of the levansucrase for sucrose and levans of low molecular weight were determined, and the yield of levan reached 90% in the presence of the former initiator. The enzyme was shown to consist of a single polypeptide chain, with L-lysine as the *N*-terminus, and appeared to be identical with the levansucrase obtained from another strain of *B. subtilis*.

Pectinesterases.—The purification of pectinesterase from *Citrus natsudaidai* by ion-exchange chromatography and gel filtration has been reported.[384] The enzyme exhibited maximum activity at pH 8 and was stable in the pH range 5—8. The kinetics, thermal stability, and composition of the enzyme were investigated.

Peroxidases.—During studies of the amino-acid sequence of horse-radish peroxidase, a tryptic glycopeptide containing 2-amino-2-deoxy-D-glucose, two histidinyl residues, and a disulphide bridge was isolated.[385]

Phosphatases.—An acid phosphatase extracted from disintegrated cells of *Candida albicans* has been purified by column chromatography to give a preparation that was homogeneous by ultracentrifugal, polyacrylamide-gel electrophoretic, and immunological analyses.[386] Molecular weights of 1.24 × 10^5, 1.15 × 10^5, and 1.31 × 10^3 were estimated from ultra-centrifugation, gel filtration, and polyacrylamide-gel electrophoresis, respectively, for the enzyme ($S_{20,w}$ = 5.51 *S*). The acid phosphatase was found to be a mannoprotein having a hexose : protein ratio of 7 : 1; preliminary evidence suggested that the mannan portion of the molecule is responsible for its antigenicity.

Sulphatases.—A comparison has been made of the arylsulphatase and cerebroside-sulphatase activities of bovine liver.[387] From studies of the kinetics, inhibition, *etc.* of the enzymes, explanations for the apparent differences between the two activities were derived. It was concluded that the two activities are manifested by a single active site on a single protein molecule.

Arylsulphatase from chicken brain was precipitated in an immobilized form on interaction with concanavalin A, indicating that the enzyme is a glycoprotein.[388] The immobilized form of the enzyme retained the catalytic

[384] M. Manabe, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1487.
[385] K. G. Welinder, *F.E.B.S. Letters*, 1973, **30**, 243.
[386] F. C. Odds and J. C. Hierholzer, *J. Bacteriol.*, 1973, **114**, 257.
[387] A. Jerfy and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **293**, 178.
[388] A. Ahmad, S. Bishayee, and B. K. Bachhawat, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 730.

activity and could be used repeatedly without significant loss of activity. Kinetic studies indicated that the bound and free enzymes possess similar properties, although slight differences were observed between their pH optima, K_m values, and activation energies. The stabilities of the free and bound enzymes to storage were comparable, and binding of the enzyme to concanavalin A increased its thermal stability.

Sulphate ester hydrolase activities present in skin fibroblasts obtained from normal subjects and from cases of Sanfilippo A, Sanfilippo B, Hurler, Hunter, and Maroteaux–Lamy syndromes have been determined and compared; the levels for each of the cases appeared to be normal.

The sulphate groups have been located (by periodate oxidation and partial acid hydrolysis) on a series of mono- and di-saccharides obtained from dermatan [^{35}S]sulphates isolated from cultured fibroblasts from normal subjects and from cases of Hurler and Hunter syndromes.[389] Dermatan sulphate from normal subjects was found to terminate in a sulphated 2-acetamido-2-deoxy-β-D-galactopyranosyl residue, whereas a sulphated L-idopyranuronosyl residue occupies the terminal, non-reducing position of dermatan sulphate from cases of Hunter, but not Hurler, syndrome. It was concluded that the two conditions can be differentiated enzymically, and that Hunter syndrome is due to a deficiency of a sulphatase specific for sulphated L-idopyranuronosyl residues.

Sulphoglucosamine Sulphamidases.—Cultured skin fibroblasts and peripheral leucocytes from patients with Sanfilippo A disease were found to be strikingly deficient in sulphoglucosamine sulphamidase activity, as measured using (*N*-[^{35}S]sulphate)heparin as substrate.[390] Since the fibroblasts possessed normal sulphate ester hydrolase activities towards oligosaccharides prepared from [^{35}S]sulphate-labelled heparan sulphate by treatment with nitrous acid, the basic defect in Sanfilippo disease is considered to arise from inactivation of a sulphoglucosamine sulphamidase. A partial deficiency of this enzyme was observed in the cells of heterozygote carriers, whereas normal levels were found for cases of Sanfilippo B, Hurler, Hunter, and Maroteaux–Lamy syndromes.

Thioglucosidases.—Ultrasonic treatment has been found to release a thioglucosidase from insoluble particles of *Crambe abyssinica* seed meal.[391] The crude enzyme was optimally activated by ascorbate, required the presence of a reducing agent for maintenance of stability, and was inhibited by 4-chloromercuribenzenesulphonate. Gel filtration of an extract of the seed meal gave two separate fractions, with 80% of the original activity occurring in the fraction of lower molecular weight (1.1×10^5). The pH optimum of this enzyme was found to be pH 9, whereas that for the crude enzyme was pH 10. Both the crude enzyme and the purified fractions

[389] I. Sjöberg, L.-Å. Fransson, R. Matalon, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1125.
[390] H. Kresse, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1111.
[391] H. L. Tookey, *Canad. J. Biochem.*, 1973, **51**, 1305.

produced goitrin from epi-progoitrin, but, in the presence of Fe^{2+} ions, 1-cyano-2-hydroxybut-3-ene was produced. Ferrous ions gave rise to substrate inhibition at high levels of epi-progoitrin.

Index of Enzymes Referred to in Chapter 6*

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
endo-α-Acetamidodeoxy-galactosidase			405
β-Acetamidodeoxy-galactosidase	2-acetamido-2-deoxy-β-D-galactoside acetamidodeoxygalactohydrolase	3.2.1.53	363
α-Acetamidodeoxy-glucosidase	2-acetamido-2-deoxy-α-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.50	363
αβ-Acetamidodeoxy-glucosidase			363
β-Acetamidodeoxy-glucosidase	2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	363
β-Acetamidodeoxy-glucosylamine-asparagine amidase	see 4-L-aspartylglycosylamine amidohydrolase		464
β-Acetamidodeoxy-hexosidase	2-acetamido-2-deoxy-β-hexoside acetamidodeoxyhexohydrolase	3.2.1.52	363
Acetylcholinesterase	acetylcholine hydrolase	3.1.1.7	463
N-Acetyl-lactosamine synthetase		3.4.1.22	463
N-Acetylneuraminate lyase	*N*-acetylneuraminate pyruvate-lyase	4.1.3.3	463
Acid phosphatase	orthophosphoric monoester phosphohydrolase (acid optimum)	3.1.3.2	466
Adenylate cyclase	ATP pyrophosphate-lyase	4.6.1.1	463
Agarase			406
Alginase	poly-(1,4-β-D-mannuronide) glycanohydrolase	3.2.1.16	406
Alginate lyase	poly-(1,4-β-D-mannuronide) lyase	4.2.2.3	406
α-Amylase	1,4-α-D-glucan glucanohydrolase	3.2.1.1	406
β-Amylase	1,4-α-D-glucan maltohydrolase	3.2.1.2	417
Amylo-1,6-glucosidase	dextrin 6-α-glucosidase	3.2.1.33	419
α-L-Arabinofuranosidase	α-L-arabinofuranoside arabinohydrolase	3.2.1.55	373
L-Arabinose isomerase	L-arabinose ketol isomerase	5.3.1.4.	459
β-L-Arabinosidase			373
Arylsulphatase	aryl sulphate sulphohydrolase	3.1.6.1	466
4-L-Aspartylglycosyl-amine amidohydrolase	2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucosylamine glucosylamidohydrolase	3.5.1.37	464
Bromelain (stem)		3.4.22.4	461
Carbohydrate epimerases			459
Carbohydrate isomerases			459
Carbohydrate oxidases			460
Carrageenase			420
Cellobiosidase			424

* See the Introduction (Part II, Chapter 1).

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
Cellulase	1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase	3.2.1.4	420
Cerebroside sulphatase	cerebroside-3-sulphate 3-sulphohydrolase	3.1.6.8	466
Ceruloplasmin			464
Chitinase	poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)] glycanohydrolase	3.2.1.14	425
Deoxyribonuclease I	deoxyribonucleate 5'-oligonucleotidohydrolase	3.1.4.5	462
Dextranase	1,6-α-D-glucan 6-glucanohydrolase	3.2.1.11	425
Dextrin 1,6-glucosidase	see amylo-1,6-glucosidase		419
β-Fructofuranosidase	β-D-fructofuranoside fructohydrolase	3.2.1.26	374
α-D-Fucosidase	α-D-fucoside fucohydrolase		375
α-L-Fucosidase	α-L-fucoside fucohydrolase	3.2.1.51	375
β-D-Fucosidase	β-D-Fucoside fucohydrolase	3.2.1.38	375
Galactanase			427
Galactose oxidase	D-galactose:oxygen 6-oxidoreductase	1.1.3.9	460
α-Galactosidase	α-D-galactoside galactohydrolase	3.2.1.22	377
β-Galactosidase	β-D-galactoside galactohydrolase	3.2.1.23	377
β-Galactosylceramidase	D-galactosyl-*N*-acylsphingosine galactohydrolase	3.2.1.46	377
Galactosylgalactosyl-glucosylceramidase	D-galactosyl-D-galactosyl-D-glucosyl-ceramide galactohydrolase	3 2.1.47	377
endo-β-1,3-Glucanase	1,3-β-D-glucan glucanohydrolase	3.2.1.39	428
endo-β-1,6-Glucanase	1,6-β-D-glucan glucanohydrolase	3.2.1.75	429
Glucanase (miscellaneous)			430
4-α-Glucanotransferase	1,4-α-D-glucan:1,4-α-D-glucan 4-α-glycosyltransferase	2.4.1.25	419
Glucoamylase	1,4-α-D-glucan glucohydrolase	3.2.1.3	432
Glucose isomerase	D-glucose ketol-isomerase	5.3.1.18	459
Glucose oxidase	β-D-glucose:oxygen 1-oxidoreductase	1.1.3.4	460
α-Glucosidase	α-D-glucoside glucohydrolase	3.2.1.20	389
β-Glucosidase	β-D-glucoside glucohydrolase	3.2.1.21	389
exo-β-1,3-Glucosidase	1,3-β-D-glucan glucohydrolase	3.2.1.58	435
exo-β-1,4-Glucosidase	1,4-β-D-glucan glucohydrolase	3.2.1.74	435
exo-α-1,6-Glucosidase	1,6-α-D-glucan glucohydrolase	3.2.1.70	435
β-Glucosylceramidase	D-glucosyl-*N*-acylsphingosine glucohydrolase	3.2.1.45	390
β-Glucuronidase	β-D-glucuronide glucuronohydrolase	3.2.1.31	396
Hexose oxidase	D-hexose:oxygen 1-oxidoreductase	1.1.3.5	461
Hyaluronate lyase	hyaluronate lyase	4.2.2.1	435
Hyaluronidase	hyaluronate 4-glycanohydrolase	3.2.1.35	435
α-L-Iduronidase	α-L-iduronide iduronohydrolase	3.2.1.76	398
Inulase	see inulinase		438
Inulinase	2,1-β-D-fructan fructanohydrolase	3.2.1.7	438
Isoamylase	glycogen 6-glucanohydrolase	3.2.1.68	438
Isopullulanase	pullulan 4-glucanohydrolase	3.2.1.57	438
α-Lactalbumin		2.4.1.22	465
Lactose synthetase	UDP galactose:D-glucose 4-β-galactosyltransferase	2.4.1.22	465

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
Laminarinase	1,3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	3.2.1.6	438
Levansucrase	sucrose:2,6-β-D-fructan 6-β-fructosyltransferase	2.4.1.10	466
Limit dextrinase			439
Luteanase	see *endo*-β-1,6-glucanase and *exo*-β-1,6-glucosidase		429, 435
Lysozyme	mucopeptide *N*-acetylmuramoyl-hydrolase	3.2.1.17	439
exo-Lysozyme			439
endo-β-1,4-Mannanase	1,4-β-D-mannan mannanohydrolase	3.2.1.78	451
Mannanase (miscellaneous)			452
α-Mannosidase	α-D-mannoside mannohydrolase	3.2.1.24	399
β-Mannosidase	β-D-mannoside mannohydrolase	3.2.1.25	399
Neuraminidase	acylneuraminyl hydrolase	3.2.1.18	403
Oligo-1,3-glucosidase	see *endo*-β-1,3-glucanase		428
Pectate lyase	poly(1,4-α-D-galacturonide) lyase	4.2.2.2	452
Pectin lyase	poly(methoxygalacturonide) lyase	4.2.2.10	453
Pectinesterase	pectin pectyl-hydrolase	3.1.1.11	466
Peptidase	see proteinase		461
Peroxidase	donor:hydrogen-peroxide oxidoreductase	1.11.1.7	466
Phosphatase			466
Polygalacturonase	poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15	454
exo-Polygalacturonase	poly(1,4-α-D-galacturonide) galacturonohydrolase	3.2.1.67	456
Polygalacturonate lyase	see pectate lyase and pectin lyase		452, 453
Proteinase			461
Pullulanase	pullulan 6-glucanohydrolase	3.2.1.41	457
Quercitrinase	quercitrin 3-rhamnohydrolase	3 2.1.66	403
Rhamnanase			458
α-L-Rhamnosidase	α-L-rhamnoside rhamnohydrolase	3.2.1.40	402
β-L-Rhamnosidase	β-L-rhamnoside rhamnohydrolase	3.2.1.43	402
Ribonuclease I	ribonucleate 3′-pyrimidino-oligonucleotidohydrolase	3.1.4.22	462
Sialidase*			403
Sulphatase			466
Sulphoglucosamine sulphamidase	2-sulphamido-2-deoxy-D-glucose sulphamidase	3.10.1.1	467
Thioglucosidase	thioglucoside glucohydrolase	3.2.3.1	467
Thrombin		3.4.21.5	462
Trehalase	αα-trehalase glucohydrolase	3.2.1.28	458
Xylanase (miscellaneous)			458
α-Xylosidase	α-D-xyloside xylohydrolase		405
β-Xylosidase	β-D-xyloside xylohydrolase	3.2.1.37	405
Zosterinase			402, 454, 456

* Sialidase is used generally to include neuraminidase and unspecified acylneuraminyl hydrolases.

7
Glycolipids and Gangliosides

BY R. J. STURGEON

Introduction

Books or reviews have been published covering various aspects of glycolipids and gangliosides as follows: lysosomes and storage diseases,[1] alterations of lipid metabolism in tumorigenic, virus-transformed cells,[2] and glycolipids of the nervous system.[3]

Animal Glycolipids and Gangliosides

A new procedure for the extraction, purification, and fractionation of brain gangliosides is claimed to achieve a more complete extraction of the gangliosides, with neither concurrent solubilization of glycoprotein nor significant degradation of the gangliosides, compared with conventional extraction procedures.[4] Losses of gangliosides have been reported to occur on dialysis when the molecules are present at low concentrations.[5] The galactose oxidase–sodium borotritide system has been used to label specifically the terminal 2-acetamido-2-deoxy-D-galactose residues of three glycosphingolipids, namely G_{M_2}-ganglioside, asialo-G_{M_2}-ganglioside, and globoside.[6] All the compounds showed a minimum of 95% radiopurity, and generally had more than 90% of the total radioactivity located in their terminal amino-sugar moieties, suggesting that these compounds would be well suited as substrates for studies of specific sphingolipid *N*-acetylgalactosaminidases. D-[6-^{3}H]Galactosyl-D-glucosylceramide has been prepared by oxidation of lactosylceramide with galactose oxidase, followed by sodium borotritide reduction of the oxidized product.[7] A water-soluble, radioactive contaminant that appeared on storage was identified as [^{3}H]lactose by both chromatographic and carrier-dilution techniques. A series of monoglycosylceramides, having β-D-galactopyranosyl units

[1] 'Lysomes and Storage Diseases', ed., H. G. Hers and F. Van Hoof, Academic Press, New York, 1973.
[2] R. O. Brady in 'Membranes and Viruses in Immunopathology', ed. S. B. Day and R. A. Good, Academic Press, New York, 1973.
[3] 'Glycolipids, Glycoproteins, and Mucopolysaccharides of the Nervous System', ed. V. Zambotti, G. Tettamanti, and M. Arrigoni, *Adv. Exp. Med. Biol.*, 1972, **25**, 1.
[4] G. Tettamanti, F. Bonali, S. Marchesini, and V. Zambotti, *Biochim. Biophys. Acta*, 1973, **296**, 160.
[5] J. N. Kanfer and C. Spielvogel, *J. Neurochem.*, 1973, **20**, 1483.
[6] Y. Suzuki and K. Suzuki, *J. Lipid Res.*, 1972, **13**, 687.
[7] R. A. Mumford, S. S. Raghavan, D. B. Rhoads, and J. N. Kanfer, *Lipids*, 1973, **8**, 238.

linked to different ceramides, has been studied by high-resolution mass spectrometry of the acetylated derivatives.[8] The ^{1}H n.m.r. spectra of fully acetylated sphingosine, dihydrosphingosine, 1-*O*-β-D-galactopyranosyl-(2*S*,3*R*)-2-tetra-acosanoylamido-*trans*-octadec-4-ene-1,3-diol (cerasine), and 1-*O*-β-D-galactopyranosyl-(2*S*,3*R*)-2-(2′-hydroxytetra-acosanoyl)-amido-*trans*-octadec-4-ene-1,3-diol (phrenosine) have been measured.[9] The relative chemical shifts of various protons in [^{2}H]chloroform, [$^2H_6$]-acetone, and [$^2H_6$]benzene were sufficiently different to permit configurational and conformational information to be derived from partial, first-order analyses. By a double-diffusion, precipitation-in-gel technique, isolated cholera toxin, as well as its natural toxoid, have been shown to be fixed and precipitated by ganglioside G_{M1}, but not by such related glycolipids as G_{M3}, G_{M2}, G_{M1}-Glc*N*Ac, G_{D1a}, G_{D1b}, G_{T1}, globoside, G_{A1}, or tetrahexoside-Glc*N*Ac.[10] The critical region in G_{M1} for toxin fixation was identified as D-Gal → D-Gal*N*Ac → D-Gal[Neu*N*Ac], and it was postulated that this could be the tissue-receptor structure for the cholera toxin. Inactivation of this toxin caused by high concentrations of G_{A1} and G_{D1a} was considered to be due to some affinity of the toxin for neutral carbohydrate chains identical to that of G_{M1} or, in the case of G_{D1a}, by an *in vivo* conversion into G_{M1} by the action of sialidase.[11]

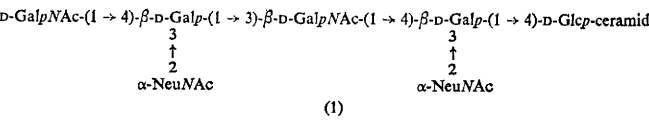
The effect of the ionic strength of the medium on the activity of *Vibrio cholerae* sialidase towards mammalian-brain gangliosides has been studied.[12] Strong electrolytes reversibly inhibited enzymic activity, and it was concluded that interaction of the enzyme with monovalent ions may conformationally govern the availability of the catalytic site. Columns of agarose derivatives containing covalently attached gangliosides quantitatively adsorbed [^{125}I]-labelled cholera toxin from chromatographed samples.[13] The most effective derivatives were found to be those in which the gangliosides are attached to macromolecules [albumin, poly-(L-lysine-DL-alanine) copolymers] that are covalently linked to agarose. Soluble ganglioside polymers, prepared by covalently attaching the glycolipids to branched copolymers of lysine and alanine, were able to prevent the binding of the [^{125}I]-labelled toxin to liver membranes, in addition to completely blocking the lipolytic activity of cholera toxin on fat cells. It was suggested that these polymeric ganglioside derivatives might prove useful in the management of the manifestations of clinical cholera. Radioactively labelled G_{A2} (2-acetamido-2-deoxy-β-D-1-[^{14}C]galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide) has been used in a study of the substrate specificity of hexosaminidases A and B from liver.[14]

[8] B. A. Ånderson, K.-A. Karlsson, I. Pascher, B. E. Samuelson, and G. O. Steen, *Chem. Phys. Lipids*, 1972, **9**, 89.
[9] M. Martin-Lomas and D. Chapman, *Chem. Phys. Lipids*, 1973, **10**, 152.
[10] J. Holmgren, I. Lönnroth, and L. Svennerholm, *Infection and Immunity*, 1973, **8**, 208.
[11] J. Holmgren, I. Lönnroth, and L. Svennerholm, *Scand. J. Infect. Dis.*, 1973, **5**, 77.
[12] V. Lipovac, N. Barton, and A. Rosenberg, *Biochemistry*, 1973, **12**, 1858.
[13] P. Cuatrecasas, I. Parikh, and M. D. Hollenberg, *Biochemistry*, 1973, **12**, 4253.
[14] D. A. Wenger, S. Okada, and J. S. O'Brien, *Arch. Biochem. Biophys.*, 1972, **153**, 116.

Ceramide lactoside was found to inhibit the hydrolysis of ceramide trihexoside at all concentrations by a purified ceramide trihexoside α-galactosidase from human liver.[15] Ceramide digalactoside stimulated the hydrolysis of the ceramide trihexoside at low concentrations, but inhibited hydrolysis of the lipid at high concentrations. G_{M1}-ganglioside has been hydrolysed by two forms of 'acid' methylumbelliferyl β-galactosidase.[16] The two isoenzymes were significantly different in substrate saturation kinetics. One of the enzymes was insensitive to G_{M2}-ganglioside, whereas the other was inhibited by this compound; these observations are consistent with the hypothesis that G_{M1}-ganglioside and its analogues act as modifiers of the latter, but not the former, enzyme.

A heat-stable, non-dialysable preparation obtained from crude β-hexosaminidase from human liver was found to stimulate the hydrolysis of G_{M2}-ganglioside by β-hexosaminidase A.[17] Extensive purification or ageing tended to reduce the capacity of the enzyme to hydrolyse this ganglioside, even in the presence of the preparation. The results explain why hexosaminidase A was previously reported to hydrolyse G_{M2}-ganglioside only with great difficulty, and also relate the storage of the ganglioside to the absence of β-hexosaminidase A in the classical form of Tay–Sachs disease. In one case of Krabbe's disease, an analysis of the cerebrosides, sulphatides, and gangliosides was carried out.[18] The accumulation of galactosyl-α-(1 → 4)-galactosylceramide in certain organs in Fabry's disease has been ascribed to an alteration of a specific galactosyl-α-(1 → 4)-galactosyl:galactosyl hydrolase.[19] An *in utero* diagnosis of Sandhoff's disease, which is caused by an error of glycosphingolipid metabolism resulting from deficient activities of β-*N*-acetylhexosaminidases A and B, has been reported.[20] This disease is characterized by neural and visceral accumulations of G_{M2}-ganglioside, asialo-G_{M2}-ganglioside, and globoside, and it was demonstrated that the pathological glycosphingolipids accumulate in uncultured amniotic cells. Altered levels of tissue glycoproteins, gangliosides, glycosaminoglycans, and lipids have been reported in a case of Neimann–Pick's disease.[21] The concentrations of G_{M3}-, G_{M2}-, and G_{D3}-gangliosides were found to be elevated in brain tissue, while there were increased levels of G_{D3}-ganglioside and lactosylceramide in the liver. A novel disialylpentahexosylceramide has been isolated from human infant brain.[22] The ganglioside was resistant to attack by sialidase,

[15] M. W. Ho, *Biochem. J.*, 1973, **133**, 1.
[16] M. W. Ho, P. Cheetham, and D. Robinson, *Biochem. J.*, 1973, **136**, 351.
[17] Y. T. Li, M. Y. Mazzotta, C. C. Wan, R. Orth, and S. C. Li, *J. Biol. Chem.*, 1973, **248**, 7512.
[18] B. Berra, E. G. Brunngraber, V. Aguilar, A. Aro, and V. Zambotti, *Clinica Chim. Acta*, 1973, **47**, 325.
[19] C. A. Mapes and C. C. Sweeley, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 1317.
[20] R. J. Desnick, W. Krivit, and H. L. Sharp, *Biochem. Biophys. Res. Comm.*, 1973, **15**, 20.
[21] E. G. Brunngraber, B. Berra, and V. Zambotti, *Clinica Chim. Acta*, 1973, **48**, 173.
[22] L. Svennerholm, J.-E. Månsson, and Y. T. Li, *J. Biol. Chem.*, 1973, **248**, 740.



(1)

but the structure (1) was identified by use of partial acid hydrolysis, sequential hydrolysis with specific glycosidases, and methylation analysis.

Quantitative analyses of urinary glycolipids showed only small amounts of glucosyl-, lactosyl-, trihexosyl-, and tetrahexosyl-ceramides in cases of Fabry's disease.[23] In cases of late infantile and late adult metachromatic leukodystrophy, there was a high excretion of galactosylsulphatide and a low excretion of lactosylsulphatide. Differences in the patterns of trihexosyl- and digalactosyl-ceramides were observed between male patients and subclinically ill, female, heterozygote carriers with Fabry's disease. Preliminary studies have indicated the presence of material similar to glucosylsphingosine in the spleen of cases of Gaucher's disease, whereas this material was not detected in normal and control spleen tissues.[24] Isolation and purification of a low-density, glucocerebroside-rich material from the spleen of a patient with Gaucher's disease have shown that it possesses a more complex structure than earlier indicated.[25] A glycolipid found in abnormal levels in biopsy specimens from the spleen and liver of a patient with lipidosis has been identified as glucocerebroside.[26] L-Fucose-rich glycolipids and glycoproteins were found to increase markedly in liver-biopsy tissue when α-fucosidase activity is absent.[27] The sugar moieties of sialoglycosphingolipids from human spleen, kidney, and liver, and from bovine spleen, kidney, liver, and udder have been isolated and characterized.[28] The structure of ganglioside $G_{Lntet1a}$ was confirmed as [3⁴]-sialyl-lactoneotetrosylceramide (2). Two newly isolated gangliosides have been shown to be [6⁴]-sialyl-lactoneotetrosylceramide (3) (from spleen and kidney) and [3²]-sialyl-[2⁴]-fucosylgangliotetrosylceramide (4) (from bovine liver). The carbohydrate moiety of the latter ganglioside exhibited blood-group H activity, inhibiting the agglutination of O(H)-erythrocytes by the lectin of *Ulex europaeus*.

A sialogalactosylceramide (G_7) was found to be a major component of human myelin.[29] From the results of fractionation and identification of the separated gangliosides, it was suggested that a metabolic relationship exists between this ganglioside and either myelin cerebroside or myelin sulphatide. A monosialotetraglycosylceramide isolated from human

[23] H. Pilz, D. Müller, and I. Linke, *J. Lab. Clin. Med.*, 1973, **81**, 7.
[24] S. S. Raghavan, R. A. Mumford, and J. N. Kanfer, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 256.
[25] R. H. Glen and R. E. Lee, *Arch. Biochem. Biophys.*, 1973, **156**, 626.
[26] P. Borri, R. P. Bertinelli, and L. Bingalia, *Boll. Soc. Ital. Biol. Sper.*, 1972, **48**, 401.
[27] I. Matsuda, S. Arashima, M. Anakura, A. Ege, and I. Hayata, *Tohoku J. Exp. Med.*, 1973, **109**, 41.
[28] H. Wiegandt, *Z. physiol. Chem.*, 1973, **354**, 1049.
[29] R. W. Ledeen, R. K. Yu, and L. F. Eng, *J. Neurochem.*, 1973, **21**, 829.

plasma, red cells, muscle, brain, and peripheral nerve has been shown to contain 2-acetamido-2-deoxy-D-glucose.[30] Treatment with sialidase resulted in the formation of a tetraglycosylceramide, which was also found as a native constituent of muscle tissue. Sequential hydrolysis with specific glycosidases and methylation analysis indicated the structure (2) for the sialotetraglycosylceramide.

Two forms of ceramide trihexosidase (A1 and A2) have been isolated from human plasma and were shown to exhibit different activities in the presence of digalactosylceramide and a trisaccharide derived from the lipid substrate.[31] Sulphatides of blood plasma and red corpuscles have been separated using silica gel and ion-exchange chromatographic techniques.[32] The constituent sugars of the sulphatides were identified as D-glucose and D-galactose. Blood sulphatides of both rat and man resemble cerebrosides in structure insofar as they are both ceramide monoglucosides. The glycolipid (2) and its asialo-derivative have been isolated from human blood-group A stroma, and they were also found in blood-group B erythrocytes and red-cell membranes (irrespective of blood-group activity).[33] The major ganglioside from human red cells has been isolated and characterized as (2), and it was noted that the linkage of the terminal sialoyl residue is labile to attack in the intact cell.[34] A glycosphingolipid isolated from polymorphonuclear leucocytes from the urine of a patient with a urinary-tract infection corresponded to the asialoganglioside. On the basis of the molar ratios of sugars, studies of permethylated products, and the action of specific glycosidases, identifications were made of four neutral glycosphingolipids of human platelets.[35] Platelets were found to resemble leucocytes, liver, and spleen in having lactosylceramide and hematoside as the principal neutral and acidic glycosphingolipids. D-Galactosyl and 2-acetamido-2-deoxy-D-galactosyl residues in glycolipids and glycoproteins on the external surfaces of human erythrocytes have been labelled with tritium by means of the galactose oxidase–sodium borotritide system.[36] The labelling patterns of normal adult cells differed greatly from foetal cells, and were significantly altered when cell surfaces were modified with proteases and neuraminidase. The results indicated that the carbohydrate moieties of two glycolipids (globoside and ceramide trihexoside, but not ceramide dihexoside and ceramide monohexoside) and at least three glycoproteins are exposed to the external environment. The entrapment of β-glucosidase and β-galactosidase inside erythrocytes by rapid haemolysis of the cells in the presence of these enzymes has been reported.[37]

[30] Y.-T. Li, J.-E. Månsson, M.-T. Vanier, and L. Svennerholm, *J. Biol. Chem.*, 1973, **248**, 2634.
[31] C. A. Maples, C. H. Suelter, and C. C. Sweeley, *J. Biol. Chem.*, 1973, **248**, 2471.
[32] M. Petit, M. C. Chamblier, and J. Polonovski, *Compt. rend.*, 1972, **275**, *D*, 3001.
[33] S. Ando, K. Kon, M. Isobe, and T. Yamakawa, *J. Biochem.* (*Japan*), 1973, **73**, 893.
[34] J. R. Wherrett, *Biochim. Biophys. Acta*, 1973, **326**, 63.
[35] R. V. P. Tao, C. C. Sweeley, and G. A. Jamieson, *J. Lipid Res.*, 1973, **14**, 16.
[36] C. G. Gahmberg and S. I. Hakomori, *J. Biol. Chem.*, 1973, **248**, 4311.
[37] G. M. Ihler, R. H. Glew, and F. W. Schnure, *Proc. Nat. Acad. Sci., U.S.A.*, 1973, **70**, 2663.

The size of proteins that can be bound in this way is critical, although enzymes having a molecular weight of 1.8×10^5 were rapidly entrapped; it was suggested that this technique could provide a useful approach to the problem of replacing enzymes in certain diseases (*e.g.* Gaucher's disease).

A blood-group A-active ceramide hexasaccharide lacking 2-acetamido-2-deoxy-D-glucose has been isolated from stomach mucosa.[38] Partial acid hydrolysis, sequential hydrolysis with glycosidases, and methylation analysis were in accord with the structure (5) for this glycolipid.

Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

(2)

Neu*N*Ac-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

(3)

L-Fuc-(1 → 2)-β-D-Gal*p*-(1 → 3)-D-Gal*p*N*A*c-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

3 ↑ 2 Neu*N*Ac

(4)

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

2 ↑ 1 α-L-Fuc

(5)

Four variants of the blood-group A-active glycolipid (A^a, A^b, A^c, and A^d) have been isolated from lipids extracted from the membrane of human erythrocytes.[39] From the results of sugar analyses and identification of the derived methylated alditol acetates using g.l.c.–mass spectrometry, tentative structures (6)—(8) for three of the variants, A^a, A^b, and A^c, respectively,

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

2 ↑ 1 L-Fuc; *n*

(6)

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

2 ↑ 1 Fuc; *n*

(7)

D-Gal*N*Ac-(1 → 3)-D-Gal-(1 → 4)-D-Glc*N*Ac-(1 → 3)-D-Gal-(1 → 4)-[D-Glc*N*Ac-(1 → 3)-D-Gal]-(1 → 4)-D-Glc-ceramide

2 ↑ 1 L-Fuc; 6 ↑ 1 D-*Gal*N*Ac*...D-Gal-(1 → 4)-D-Glc*N*Ac; *n*

2 ↑ 1 L-*Fuc*

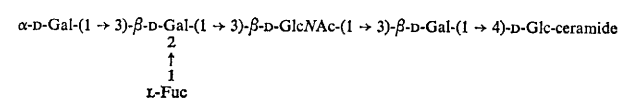
(8)

[38] B. L. Slomiany, A. Slomiany, and M. I. Horowitz, *Biochim. Biophys. Acta*, 1973, **326**, 224.

[39] S. I. Hakomori, K. Stellner, and K. Watanabe, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1061.

have been suggested. The variant A^c was considered to be a mixture of glycolipids, with or without the terminal sugar residues shown in italics [see (8)], since methylation analysis indicated that some terminals of the branched chain are D-galactose residues and some are (1 → 2)-linked L-fucose residues, but only a small number possess the complete unit. Variant A^d appears to have a structure similar to A^c, but with a larger proportion of 2-acetamido-2-deoxy-D-glucosyl residues and with additional branching.

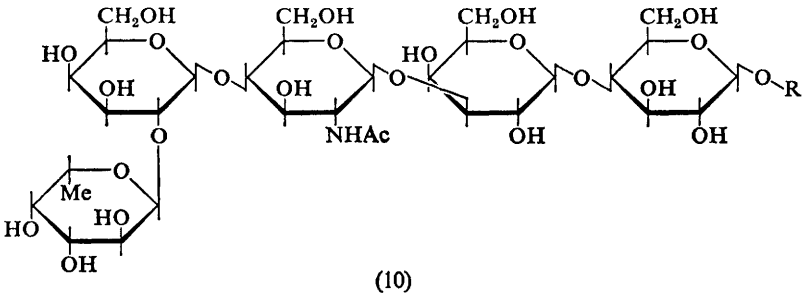
A series of glycolipids has been extracted from group-A erythrocytes and purified by column chromatography.[40] One of the glycolipids was shown to have the same carbohydrate composition as globoside I and to contain the sequence D-Gal → D-Glc*N*Ac → D-Gal → D-Glc, whereas another contained D-glucose, D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, and L-fucose (1 : 2 : 1 : 1 : 1), and inhibited anti-A haemagglutinin. A purified glycolipid isolated from blood-group A erythrocytes was shown to contain L-fucose, D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose.[41] Smith degradation caused destruction of the L-fucose and 2-amino-2-deoxy-D-galactose residues, but the reactivities to both wheat-germ agglutinin and carcinoembryonic antigen were intensified after degradation, suggesting that both activities reside on the internal structure of carbohydrate chains composed of 2-acetamido-2-deoxy-D-galactose (2 moles) and D-galactose. In addition to ceramide trihexoside and ceramide digalactoside, a ceramide hexasaccharide accumulated in the pancreas of a patient with Fabry's disease.[42] The hexasaccharide was isolated and identified as a blood-group B-active glycolipid having the structure (9). About 80% of the accumulated



(9)

glycolipid has a structure with D-galactose β-(1 → 3)-linked to 2-acetamido-2-deoxy-D-glucose, whereas a β-(1 → 4)-linkage occurs in the remaining molecules. L-Fucose-containing glycolipids with H and B blood-group activities, in addition to a glycolipid containing sialic acid and 2-amino-2-deoxy-D-glucose, have been isolated from human erythrocytes.[43] The sequences and linkages of carbohydrate residues in the glycolipids were determined by partial acid hydrolysis, periodate oxidation, and methylation studies, while the configurations of the anomeric linkages were assigned on the basis of optical data, i.r. spectroscopy, and enzymic and immunological

[40] S. Ando and T. Yamakawa, *J. Biochem.* (*Japan*), 1973, **73**, 387.
[41] K. Watanabe and S. I. Hakomori, *F.E.B.S. Letters*, 1973, **37**, 317.
[42] J. R. Wherrett and S. I. Hakomori, *J. Biol. Chem.*, 1973, **248**, 3046.
[43] J. Kościelak, A. Piasek, H. Górniak, A. Gardas, and A. Gregor, *European J. Biochem.*, 1973, **37**, 214.



(10)

studies. Structures (10) and (11) showed blood-group H activity and (12) showed blood-group B activity, whereas (13) was devoid of blood-group activity. Three forms of blood-group H-glycolipid (H_1, H_2, and H_3 variants) have been isolated from an extract of the membrane of human blood-group O erythrocytes.[44] The structure (10) of the H_1-glycolipid was indicated to be of the 'type 2' H-chain (see W. M. Watkins, *Science*, 1966, **152**, 172) linked to lipid. No 'type 1' H-chain was found in lipid-bound form in erythrocyte membranes. The H_2- and H_3-glycolipids were indicated to be ceramide octa- and deca-saccharides carrying the H-active, terminal α-L-fucopyranosyl-(1 → 2)-D-galactopyranosyl unit. Human blood-group O erythrocytes of secretors and non-secretors have been compared with respect to their enzymic convertibility by 2-acetamido-2-deoxy-D-galactosyl-transferases from blood-group A individuals.[45] Neither the rate nor the degree of conversion into erythrocytes reacting with anti-A sera depended significantly on the secretor status of the donor. This finding emphasizes that glycolipids, rather than glycoproteins, in the erythrocyte membrane are carriers of the serologically detectable A-antigenic sites, and are acceptors of the enzymically transferred 2-acetamido-2-deoxy-D-galactosyl residues. Forssman hapten from equine kidney has been sequentially degraded to globoside with α-*N*-acetylgalactosaminidase and then to ceramide trihexoside with β-*N*-acetylgalactosaminidase;[46] this information, in conjunction with that obtained from methylation analysis,[47] indicated the structure (14) for the hapten.

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

(14)

The binding of bivalent cations to purified gangliosides has been reported.[48] The action of *Vibrio cholerae* neuraminidase on the surface of intact cells and isolated sialolipid components of bovine grey matter has indicated that ionic interactions govern the steric availability of the

[44] K. Stellner, K. Watanabe, and S. I. Hakomori, *Biochemistry*, 1973, **12**, 656.
[45] H. Schenkel-Brunner and H. Tuppy, *European J. Biochem.*, 1973, **34**, 125.
[46] A. Makita, T. Yokoyama, and W. Takahasi, *Z. physiol. Chem.*, 1973, **354**, 1149.
[47] K. Stellner, H. Saito, and S. I. Hakomori, *Arch. Biochem. Biophys.*, 1973, **155**, 464.
[48] J. P. Behr and J. M. Lehn, *F.E.B.S. Letters*, 1973, **31**, 297.

(11)

(12)

(13)

catalytic site of the enzyme.[49] Sialolipid components of the intact cell-surface were found to be relatively inaccessible to the extracellular action of the enzyme, probably as a consequence of being so deeply localized. Thus, the release of sialic acid from the intact cells by neuraminidase is considered to take place from surface sialoglycoproteins. The monosialosyl-*N*-tetraglycosylceramide (G_{M1}) (15) has been isolated from ox

D-Gal-(1 → 3)-D-Gal*N*Ac-(1 → 4)-D-Gal-(1 → 4)-D-Glc-(1 → 1)-ceramide
3
↑
2
Neu*N*Ac
(15)

brain and subjected to stepwise degradation by specific α- and β-glycosidases obtained from ox spleen.[50] The results support previous views that the terminal D-galactosyl and penultimate 2-acetamido-2-deoxy-D-galactosyl residues are β-linked. It seems likely that the majority of internal D-galactosyl and D-glucosyl residues are also β-linked, but the presence of a small proportion of α-linkages was indicated by their susceptibility to α-galactosidase and α-glucosidase.

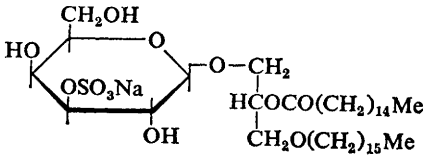
The sugar sequence of the major monosialoganglioside of ox brain has been investigated by mass spectrometry of the fully methylated and of the methylated and amide-reduced derivatives.[51] A preparation of *Clostridium perfringens* neuraminidase was found to produce asialo-derivatives on incubation with mixed ox-brain G_{M1}- and G_{M2}-gangliosides.[52] The main glycosphingolipids of bovine-kidney cortex, medulla, and papilla were shown to be glucosylceramides, galactosylceramides, and galactosylceramide sulphate esters.[53] The monoglycosylceramides of bovine kidney have been separated into sub-groups and characterized by mass spectrometry; 90% of the total cerebrosides were made up of glucosylceramide with trihydroxy-base and hydroxy-fatty-acid, and galactosylceramide with dihydroxy-base and hydroxy-fatty-acid.[54] Approximately 1—2% of the total lipids of buttermilk was characterized as ganglioside and was found to consist of neuraminosyl-lactosyl- and dineuraminosyl-lactosyl-ceramides.[55] Structural analysis indicated that the ganglioside is a neuraminosyl-(2 → 8)-neuraminosyl-(2 → 3)-lactosylceramide. Forssman hapten from sheep erythrocytes has been purified by chromatography on magnesium silicate, silica gel, and a Sephadex derivative.[56] The

[49] N. W. Barton and A. Rosenberg, *J. Biol. Chem.*, 1973, **248**, 7353.
[50] E. Werries and E. Buddecke, *European J. Biochem.*, 1973, **37**, 535.
[51] K.-A. Karlsson, *F.E.B.S. Letters*, 1973, **32**, 317.
[52] D. A. Wenger and S. Wardell, *J. Neurochem.*, 1973, **20**, 607.
[53] K.-A. Karlsson, B. E. Samuelsson, and G. O. Steen, *Biochim. Biophys. Acta*, 1973, **316**, 317.
[54] K.-A. Karlsson, B. E. Samuelsson, and G. O. Steen, *Biochim. Biophys. Acta*, 1973, **306**, 317.
[55] R. T. C. Huang, *Biochim. Biophys. Acta*, 1973, **306**, 82.
[56] B. A. Fraser and M. F. Mallette, *Immunochemistry*, 1973, **10**, 745.

homogeneous hapten migrated as either a tetrahexosyl- or a pentahexosyl-ceramide, and contained D-galactose, D-glucose, and 2-amino-2-deoxy-D-galactose (2 : 1 : 2). The glycolipids of caprine erythrocytes have been found to consist predominantly of Forssman hapten-active globoside and small amounts of glucosyl-, lactosyl-, digalactosylglucosyl-, and galactosyl-ceramides, and globoside I.[57] From investigations of the products of partial acid hydrolysis and immunochemical analysis of globoside I and the Forssman hapten, it was suggested that globoside I might be a precursor of the hapten. By analysis of the total ganglioside neuraminic acid, four major gangliosides (G_{M1}, G_{D1a}, G_{D1b}, and G_{T1}) have been identified in pig and rat brains.[58]

Evidence for a 'mannolipid' intermediate in the transfer of D-mannose from GDP-D-mannose to protein by pig-liver endoplasmic reticulum has been derived primarily from chromatographic evidence, coupled with hydrolytic data and the incorporation of [^{3}H]dolichol phosphate.[59] When pig-liver microsomes were incubated with GDP-D-[^{14}C]mannose, approximately 40% of the D-mannose was incorporated into mannolipid.[60] The chromatographic properties of the [^{14}C]mannolipid, and its lability to dilute acid and stability to dilute alkali, suggested that it contains D-mannose linked to a polyprenol *via* phosphate. Chromatographic, i.r., and ^{1}H n.m.r. spectroscopic evidence suggested the structure dolichol monophosphate D-mannose.

Three glycolipids have been isolated from pig testes and spermatozoa, the major component containing equimolar amounts of fatty acid, D-galactose, and sulphate, together with an alkyl ether of glycerol.[61] The structure (16) was proposed on the basis of methylation studies. Glycolipids with blood-group A activity have been purified from hog-stomach



(16)

mucosa.[62] Osmium-catalysed periodate oxidation released two similar, but distinct, A-active glycolipid fractions having the same carbohydrate, but different fatty-acid, compositions. Partial acid hydrolysis, enzymic digestion, and immunological assays established the sequence (17) for the A-active glycosphingolipid.

[57] T. Taketomi and N. Kawamura, *J. Biochem.* (*Japan*), 1972, **72**, 799.
[58] A. Merat and J. W. T. Dickerson, *J. Neurochem.*, 1973, **20**, 873.
[59] J. B. Richards, P. J. Evans, and F. W. Hemming, in 'The Biochemistry of the Glycosidic Linkage', ed. R. Piras and H. G. Pontis, Academic Press, New York, 1972.
[60] P. J. Evans and F. W. Hemming, *F.E.B.S. Letters*, 1973, **31**, 335.
[61] I. Ishizuka, M. Suzuki, and T. Yamakawa, *J. Biochem.* (*Japan*), 1973, **73**, 77.
[62] A. Slomiany and M. I. Horowitz, *J. Biol. Chem.*, 1973, **248**, 6232.

D-Gal*N*Ac-D-Gal-D-Glc*N*Ac-D-Gal-D-Gal-ceramide
↑
L-Fuc

(17)

The structures of glycosyl-, lactosyl-, and digalactosylglucosyl-ceramides isolated from pig-erythrocyte stroma have been determined by chemical analyses and g.l.c. of the glycosides produced on methanolysis.[63] The ultrastructure of pig-erythrocyte globoside has been examined in aqueous systems, using staining and freeze-etching techniques, and it was shown that the lipid exists in long, unbranched, filamentous tubes having a diameter of about 10 nm, which is approximately twice the length of the globoside molecule.[64]

A number of L-fucose-containing glycolipids, having similar compositions to those found in human normal and malignant gastrointestinal tissues, have been isolated from whole small intestines of individual dogs.[65] The fucolipids were divided into two classes (I and II), according to their qualitative sugar composition, with type I containing D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and L-fucose, and with type II lacking 2-amino-2-deoxy-D-galactose. Human blood-group A activity was found in significant amounts in type I fucolipid. Since only trace amounts of some fucolipids could be isolated, immunological techniques were used in their identification.[66] By use of human and rabbit antisera and *Ulex* lectin in precipitation and agglutination assays, the fucolipids were found to have human A, H-like, and Le^b-like blood-group activities. It was demonstrated with fluorescein-labelled rabbit antisera that Forssman activity is associated with the lamina propria, whereas Le^b-like activity is present in the glandular epithelium of the small intestine. A method developed for the g.l.c.–mass spectrometric identification of partially methylated amino-sugars has been applied to the analysis of a blood-group B-active ceramide pentasaccharide obtained from rabbit erythrocytes.[47] The structure of the pentasaccharide was found to be α-D-Gal-(1 → 3)-β-D-Gal-(1 → 4)-β-D-Glc*N*Ac-(1 → 3)-D-Gal-(1 → 4)-D-Glc-ceramide, contrary to a previous report in which the linkage to the amino-sugar was claimed to be Gal-(1 → 3)-Glc*N*Ac.[67] Neutral glycosphingolipids and gangliosides of cell-membrane particulate fractions from normal and allograft-rejected canine kidneys have been examined.[68] The rejected kidney showed increased concentrations of glucosyl-, galactosyl-

[63] T. Taketomi and N. Kawamura, *J. Biochem.* (*Japan*), 1972, **72**, 791.
[64] L. Pineric, D. O. Tinker, and J. Wei, *Biochim. Biophys. Acta*, 1973, **298**, 630.
[65] E. L. Smith, A. J. Bowdler, R. W. Bull, and J. M. McKibbin, *Immunology*, 1973, **25**, 621.
[66] R. N. Hiramoto, E. L. Smith, V. K. Ghanta, J. F. Shaw, and J. M. McKibben, *J. Immunol.*, 1973, **110**, 1037.
[67] T. Eto, Y. Ichikawa, K. Nishimura, S. Ando, and T. Yamakawa, *J. Biochem.* (*Japan*), 1968, **64**, 205.
[68] W. J. Esselman and J. R. Ackermann, *J. Biol. Chem.*, 1973, **248**, 7310.

glucosyl-, digalactosylglucosyl-, 2-acetamido-2-deoxygalactosyldigalactosyl-glucosyl-ceramides, Forssman hapten, and sialylgalactosylglucosylceramide, whereas lower concentrations of galactosylceramide, digalactosylceramide, and galactosylsulphatide were observed. Intravenous injection of 2-amino-2-deoxy-D-[1-^{14}C]glucose into a kidney-transplant recipient revealed a rapid incorporation of radioactivity into the amino-sugar-containing glycosphingolipids of the membrane of the rejected kidney, but not into those of the membrane of the normal kidney. The pentahexosylceramide from canine intestine and kidney was shown by enzymic hydrolysis, methylation analysis, and serological tests to be a Forssman hapten identical in structure with that from horse spleen.[69] The ganglioside patterns from rabbit-brain cortex found in nerve endings, light membranes of microsomal origin, and a myelin-rich preparation from a crude mitochondrial fraction were similar, except that the myelin-rich preparation contained higher amounts of monosialoganglioside G_{M1}.[70] As well as cerebroside and sulphatide, four other glycolipids were isolated from rabbit sciatic nerve.[71] Three were shown to be fatty-acid esters of cerebroside, and the fourth was characterized as a diacylglycerolgalactoside and its alkyl ether analogue. The *in vitro* biosynthesis of a blood group B-specific pentaglycosylceramide from UDP-D-[^{14}C]galactose and β-D-galactosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-glucosyl-(1 → 3)-β-D-galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide has been achieved using an α-galactosyltransferase from rabbit bone-marrow.[72] A hematoside, two disialogangliosides, and a trisialoganglioside have been isolated from rabbit muscle.[73]

The ganglioside pattern of the outer membranes of rat-brain synaptosomes showed the presence of monosialogangliosides; similar components, but in much higher concentrations, were contained in the myelin fraction, where 60% of the monosialoganglioside consisted of G_{M1}.[74] An enzyme that catalyses the transfer of *N*-acetylneuraminic acid from CMP-*N*-acetyl-[^{14}C]neuraminic acid to galactosyl-*N*-acetylgalactosaminyl[*N*-acetylneuraminyl]galactosylglucosylceramide (G_{M1}) in young rat brain has been described.[75] Incorporation of radioactivity was stimulated in the presence of G_{M1} and was dependent on detergent for normal activity; the radioactive product was identified as *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]galactosylglucosylceramide (G_{D1a}). A cytidine-5′-monophospho-*N*-acetylneuraminic acid:galactosyl-*N*-acetylgalactosaminylgalactosylglucosylceramide sialyltransferase was also

[69] S. J. Sung, W. J. Esselman, and C. C. Sweeley, *J. Biol. Chem.*, 1973, **248**, 6528.
[70] G. Tettamanti, A. Preti, A. Lombardo, F. Bonali, and V. Zambotti, *Biochim. Biophys. Acta*, 1973, **306**, 466.
[71] H. Singh, *J. Lipid Res.*, 1973, **14**, 41.
[72] M. Basu and S. Basu, *J. Biol. Chem.*, 1973, **248**, 1700.
[73] F. E. Lassaga, I. Albarracin de Lassaga, and R. Caputto, *J. Lipid Res.*, 1972, **13**, 810.
[74] N. F. Avrova, E. Y. Chenykaeva, and E. L. Obukhova, *J. Neurochem.*, 1973, **20**, 997.
[75] M. C. M. Yip, *Biochim. Biophys. Acta*, 1973, **306**, 298.

isolated from rat brain.[76] The product resulting from incubation of the enzyme with CMP-*N*-acetylneuraminic acid and gangliotetraose contained neuraminidase-labile neuraminic acid, and was assigned the structure (18).

Neu*N*Ac-(2 → 3)-β-D-Gal-(1 → 3)-β-D-Gal*N*Ac-(1 → 4)-β-D-Gal-(1 → 4)-D-Glc-ceramide
(18)

The quantitative pattern of galactolipids *in vitro*, compared with values obtained *in vivo*, in myelinating cultures of rat cerebellum has been reported at various stages of development.[77] It was considered that a measure of the concentration of galactolipid might serve as an indication of the presence of myelin and oligoendroglial synthetic activity during myelination *in vitro*. The proportion of glucosyl- to galactosyl-cerebroside in developing rat brain was shown to decrease rapidly as the brain matured, and is negligible in adult brain.[78] A method has been developed for the assay of an enzymic reaction, where the substrate is an 'insoluble' lipid, in the absence of detergents.[79] The metabolism of Tay–Sachs ganglioside, Gal*N*Ac-[Neu*N*Ac]Gal-Glc-ceramide(G_{M_2}), has been investigated by means of specific labelling with either [^{3}H]neuraminic acid or 2-acetamido-2-deoxy-D-[^{14}C]galactose.[80] Two possible pathways, initiated *via* G_{M_2}-sialidase or G_{M_2}-hexosaminidase, were considered for the catabolism of G_{M_2} in the brain. The products of sialidase action were identified as neuraminic acid and Gal*N*Ac-Gal-Glc-ceramide, whereas hexosaminidase yielded 2-acetamido-2-deoxy-D-galactose and Neu*N*Ac-Gal-Glc-ceramide, thus supporting the presence of two alternative pathways in mammalian brain for the catabolism of Tay–Sachs ganglioside. *In vivo* and *in vitro* experiments have demonstrated that rat-liver cells are able to synthesize a double-labelled mannolipid from [^{3}H]retinol and GDP-D-[^{14}C]mannose.[81] Biosynthesis was found to depend on ATP, Mn^{2+} ions, and retinol when the enzyme was prepared from rats deficient in vitamin A. Hydrogenolysis experiments, which released all of the tritium (as hydrocarbon) and 40% of the D-[^{14}C]mannose, in conjunction with biphasic, hydrolysis kinetics and chromatography, indicated the presence of at least two mannolipids. UDP-D-[^{14}C]glucose and UDP-D-[^{14}C]glucuronic acid were also able to form double-labelled glycolipids. When glycosphingolipids isolated from a membrane fraction of detergent-treated, rat-liver lysosomes were analysed, glucosyl-, lactosyl-, and galactosyl-galactosylglucosyl-ceramides were shown to be the predominant components of the neutral glycosphingolipid fraction.[82] *N*-Acetylneuraminyl-lactosyl-ceramide (hematoside) was the main ganglioside detected. In view of

[76] M. C. M. Yip, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 737.
[77] N. Latovitzki and D. H. Silberberg, *J. Neurochem.*, 1973, **20**, 1771.
[78] M. Hoshi, M. Williams, and Y. Kishimoto, *J. Neurochem.*, 1973, **21**, 709.
[79] S. Gatt, A. Herzl, and Y. Barenholz, *F.E.B.S. Letters*, 1973, **30**, 281.
[80] J. F. Tallman and R. O. Brady, *J. Biol. Chem.*, 1972, **247**, 7570.
[81] L. De Luca, N. Maestri, G. Rosso, and G. Wolf, *J. Biol. Chem.*, 1973, **248**, 641.
[82] R. Henning and W. Stoffel, *Z. physiol. Chem.*, 1973, **354**, 760.

possible participation of the plasma membrane in formation of the secondary lysosomal membrane, the glycosphingolipids of the two membrane fractions were compared by t.l.c. Trihexosylceramide was found to be absent from the neutral glycolipids of the plasma membrane, and the ganglioside compositions of the two membranes are entirely different. Since the composition of primary lysosomal membranes is not known, it is considered that the glycosphingolipids in secondary lysosomal membranes might be derived from the plasma membrane by detergent-induced pinocytosis; alternatively, this class of lipid could be a genuine lysosomal constituent. Extraction of glycolipids from rat-liver membranes resulted in virtually total loss of the ability to bind the enterotoxin from *Vibrio cholerae*.[83] The ability of the gangliosides to become incorporated into the membranes was shown to be a rapid and time-dependent process.[84] The evidence indicated that the gangliosides could be spontaneously incorporated into membranes in a manner that created new and stable binding sites for the cholera toxin. These sites are kinetically similar to those of the normal toxin receptors, and their interaction with the toxin led to identical biological effects. It is considered that the cholera toxin initially forms an inactive ganglioside–toxin receptor complex on the cell membrane, and that this complex is then transformed into a biologically active complex by a transition involving a spontaneous relocation of the complex within the two-dimensional structure of the membrane.[85] The biologically inactive component was reported to be a protein (choleragenoid), which is derived from the exotoxin of *V. cholerae*, and together with the cholera toxin probably forms a complex with the gangliosides on the cell surface.[86]

Incubation of liver microsomes with dolichol-D-[^{14}C]glucosyl monophosphate led to the labelling of an endogenous acceptor, which appears to be an oligosaccharide comprised of about twenty monosaccharide units bound to dolichol through a phosphate or pyrophosphate bridge.[87] The microsomal preparation also catalysed the transfer of oligosaccharides to an endogenous protein. Preliminary evidence indicated that several water-soluble products, such as D-glucose, oligosaccharides, and possibly oligosaccharides bound to amino-acids, are also formed. Various treatments of the labelled oligosaccharide-dolichol derivative afforded additional information on its structure.[88] Reduction with sodium borohydride, followed by acid hydrolysis, gave only radioactive D-glucose, indicating that this sugar is not incorporated at the reducing-end of the oligo-

[83] P. Cuatrecasas, *Biochemistry*, 1973, **12**, 3547.
[84] P. Cuatrecasas, *Biochemistry*, 1973, **12**, 3558.
[85] P. Cuatrecasas, *Biochemistry*, 1973, **12**, 3567.
[86] P. Cuatrecasas, *Biochemistry*, 1973, **12**, 3577.
[87] A. J. Parodi, N. H. Behrens, L. F. Leloir, and H. Carminatti, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3268.
[88] A. J. Parodi, R. Staneloni, A. I. Cantarella, L. F. Leloir, N. H. Behrens, H. Carminatti, and J. A. Levy, *Carbohydrate Res.*, 1973, **26**, 393.

saccharide. The percentage of radioactivity, liberated as formic acid after periodate oxidation, indicated that more than one residue of D-glucose is incorporated, with at least one of these residues within the oligosaccharide chain. Treatment of the otherwise neutral oligosaccharide with alkali liberated two positively charged derivatives, presumed to be hexosamine residues. A synthetic analogue of glucocerebroside was found to inhibit the glucosidase in rat spleen that hydrolyses glucocerebroside.[89] The mode of action appeared to be competitive, probably aided by tight binding of the amino-group to a carboxy-group near the active site of the enzyme. It was suggested that the compound may be useful for inducing an animal model of Gaucher's disease. The ceramide trihexoside synthesized by rat-spleen homogenates could be degraded by α-galactosidases, but not by β-galactosidases; this trihexoside was shown to have the same terminal α-galactosyl configuration as the corresponding lipid characterized from various human tissues.[90] The *in vitro* biosynthesis of a trihexosylceramide, containing D-galactose and D-glucose (2 : 1), was demonstrated in the presence of galactosyltransferases from rat spleen and bone marrow.[91, 92] The product was shown by methylation analysis to contain two D-galactosyl residues in (1 → 3)-linkage, and, together with other evidence, supported the structure (19).[93]

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
(19)

A ceramide tetrasaccharide from rat kidney was shown to possess the same composition [(D-galactose, D-glucose, 2-acetamido-2-deoxy-D-galactose (2 : 1 : 1)] as the corresponding tetrasaccharide obtained from human erythrocytes.[94] Sequential degradation by β-galactosaminidase and α- and β-galactosidases indicated that the two tetrasaccharides have identical sequences of sugars. However, methylation and periodate oxidation revealed that the penultimate D-galactose unit in rat-kidney glycolipid is (1 → 3)-linked, as illustrated in (20), compared with a (1 → 4)-linkage in the human-erythrocyte glycolipid.

β-D-Gal*N*Ac-(1 → 3)-α-D-Gal-(1 → 3)-β-D-Gal-(1 → 4)-β-D-Glc-ceramide
(20)

The major glycosphingolipids of rat small intestinal mucosa are monohexosylceramide, trihexosylceramide, and ganglioside containing *N*-glycolylneuraminic acid.[95] Other fractions were shown to contain

[89] J. S. Erickson and N. S. Radin, *J. Lipid Res.*, 1973, **14**, 133.
[90] I. Bensaude, M. Philippart, and J. Hildebrand, *Biochem. Med.*, 1972, **6**, 522.
[91] P. Stoffyn, A. Stoffyn, and G. Hauser, *Trans. Amer. Soc. Neurochem.*, 1972, **3**, 125.
[92] P. Stoffyn, A. Stoffyn, and G. Hauser, *J. Biol. Chem.*, 1973, **248**, 1920.
[93] P. Stoffyn, A. Stoffyn, and G. Hauser, *Biochim. Biophys. Acta*, 1973, **306**, 283.
[94] B. Siddiqui, J. Kawanami, Y. T. Li, and S. I. Hakomori, *J. Lipid Res.*, 1972, **13**, 657.
[95] G. G. Forstner and J. R. Wherrett, *Biochim. Biophys. Acta*, 1973, **306**, 446.

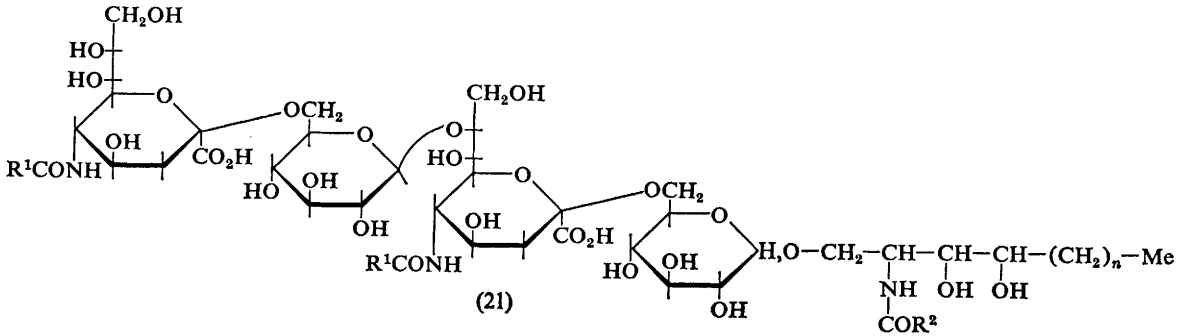
L-fucose. The distribution of the glycosphingolipids is proportionally similar in the plasma membrane, except for the appearance of a substantial level of dihexosylceramide. Preparations of rat testes have been found to contain a sulphotransferase that catalyses the transfer of sulphate from 3′-phosphoadenosine 5′-phosphosulphate to monoalkylmonoacylglyceryl-galactoside.[96] Cytolipin R, a ceramide tetrahexoside hapten from rat lymphosarcoma, has been sequentially hydrolysed with specific glycosidases to reveal the anomeric configurations of the glycosidic bonds.[97] From results of g.l.c.–mass spectrometry of the derived methylated alditol acetates, the structure was found to be identical to that of the rat-kidney glycolipid (20). Cytolipin K (globoside I) differs in having an α-D-galactosyl-(1 → 4)-D-galactosyl internal linkage, which possibly accounts for the immunological difference between cytolipins K and R. An altered pattern of gangliosides in rat mammary carcinoma has been reported.[98] In this tumour, G_{M1} accumulated at the expense of disialoganglioside, and this was attributed to depression of the activity of CMP-*N*-acetylneuraminic acid:G_{M1}sialyltransferase, but at the same time greatly elevated levels of UDP-2-acetamido-2-deoxy-D-galactosyl:G_{M_2}2-acetamido-2-deoxy-D-galactosyltransferase and CMP-*N*-acetylneuraminyl:G_{M3}sialyltransferase activities were recorded. The incorporation of L-[U^{14}C]- and [1-^{3}H]-fucose into glycolipids of normal, murine leukaemia virus-infected, murine sarcoma-murine leukaemia-transformed, and feline sarcoma-leukaemia virus-transformed cell lines has been studied.[99] In each of the non-transformed cell lines, one or two closely associated L-fucosyl lipids of low chromatographic mobility and high molecular weight were predominant. But in the transformed lines there was a sharp decrease in the incorporation of L-fucose into compounds of high molecular weight and a corresponding rise in its incorporation into more mobile and simpler L-fucosyl compounds. Exogenous globoside added to the medium was taken up by NIL hamster cells and accumulated as a component of plasma membrane, as evidenced by the recovery of tritiated globoside from the membranes.[100] 1-β-D-Arabinofuranosylcytosine brought about the transformation of hamster-embryo fibroblasts, and inhibited the incorporation of *N*-acetylneuraminic acid into glycolipids of both normal and transformed, hamster-embryo cells in tissue culture.[101] Incorporation of D-[^{3}H]glucose (a precursor of sialic acid) into glycolipids was strongly inhibited, and that of *N*-acetyl-[^{14}C]neuraminic acid was completely inhibited, suggesting that the interference with DNA synthesis by 1-β-D-arabinofuranosylcytidine may not

[96] A. Knapp, M. J. Korblatt, H. Schachter, and R. K. Murray, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 179.
[97] R. Laine, C. C. Sweeley, Y. T. Li, A. Kisic, and M. M. Rapport, *J. Lipid Res.*, 1972, **13**, 519.
[98] T. W. Keenan and D. J. Morré, *Science*, 1973, **182**, 935.
[99] S. Steiner, P. J. Brennan, and J. L. Melnick, *Nature New Biol.*, 1973, **245**, 19.
[100] R. A. Laine and S. I. Hakomori, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1039.
[101] A. O. Hawtrey, T. Scott-Burden, P. Jones, and G. Robertson, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1282.

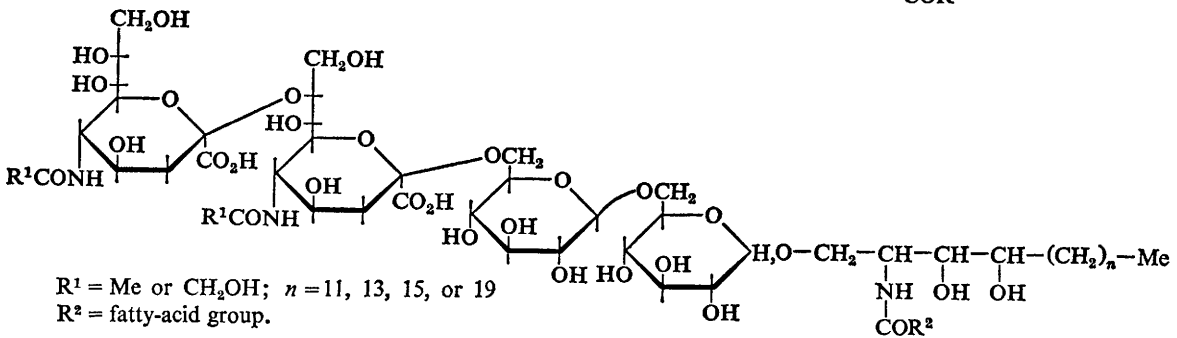
be the only factor involved in cell transformation. Density-dependent glycolipids have been shown to be present at, but not confined to, the cell surface in a hamster cell line.[102] The synthesis of glycolipid in baby hamster-kidney fibroblasts transformed by a thermosensitive mutant of polyoma virus was found to be impaired.[103] Synthesis of a trihexosylceramide in non-transformed cells was cell-density dependent, and there was little synthesis of the glycolipid at both permissive and non-permissive temperatures in transformed cells. 'Normal' glycosphingolipid synthesis was apparently not required for the control of growth exhibited by this cell line. It has been demonstrated that '2-deoxy-D-glucose' could be metabolized beyond '2-deoxy-D-gluconic acid 6-phosphate' in both normal and Simian virus 40 (SV 40)-transformed hamster cells.[104] Short-term labelling experiments revealed that both '2-deoxy-D-glucose' and 2-amino-2-deoxy-D-glucose were rapidly incorporated into glycolipid. Five neutral glycolipids, ranging from ceramide mono- to penta-saccharide, and sialyldihexosylceramide were synthesized in NIL 2 hamster cells.[105] Incorporation of [1-^{14}C]palmitate into the glycolipids gave increased labelling of the ceramide tri-, tetra-, and penta-hexosides in dense, as compared to sparse, cultures. A quantitative relationship has been found between the rate of formation of psychosine *in vitro* and the rate of accumulation of galactosylsphingosine *in vivo* in the central and peripheral nervous systems of Quaking mice.[106] Sialyltransferase activity was found to be reduced when normal mouse cells were transformed by SV 40, and hamster cell lines were transformed by polyoma virus.[107] A specific enzyme change, which is found in most DNA virus-transformed, established, mouse-cell lines, has been shown to occur in an RNA virus-induced cell transformation.[108] It is considered that this biochemical change is not due to selection for transformation by the RNA virus of cells having inherently low enzymic activity, but is more likely to be one of the direct, but delayed, consequences of viral transformation. A decrease was demonstrated in the glycolipid transferase UDP-2-acetamido-2-deoxy-D-galactose: hematoside 2-acetamido-2-deoxy-D-galactosyltransferase, an enzyme required for elongation of the carbohydrate chain of gangliosides.

The transfer of D-mannose from GDP-D-mannose to chloroform-soluble lipids by chicken-brain extracts was stimulated by the addition of dolichol phosphate, whereas analogous transfers from other sugar nucleotides occurred to a much smaller extent.[109] Embryonic chicken-brain extracts have been shown to catalyse the transfer of 2-acetamido-2-

[102] D. R. Critchley, J. M. Graham, and I. Macpherson, *F.E.B.S. Letters*, 1973, **32**, 37.
[103] S. Hammarström and G. Bjursell, *F.E.B.S. Letters*, 1973, **32**, 69.
[104] S. Steiner and M. R. Steiner, *Biochim. Biophys. Acta*, 1973, **296**, 403.
[105] D. R. Critchley and I. Macpherson, *Biochim. Biophys. Acta*, 1973, **296**, 145.
[106] V. L. Friedrich and G. Hauser, *J. Neurochem.*, 1973, **20**, 1131.
[107] W. J. Grimes, *Biochemistry*, 1973, **12**, 990.
[108] P. T. Mora, P. H. Fishman, R. H. Bassin, R. O. Brady, and V. W. McFarland, *Nature New Biol.*, 1973, **245**, 226.
[109] W. C. Breckenridge and L. S. Wolfe, *F.E.B.S. Letters*, 1973, **29**, 66.



(21)



R^1 = Me or CH_2OH; n = 11, 13, 15, or 19
R^2 = fatty-acid group.

(22)

deoxy-D-galactose from UDP-2-acetamido-2-deoxy-D-[^{14}C]galactose to a triglycosylceramide [α-D-Gal-(1 → 4)-β-D-Gal-(1 → 4)-β-D-Glc-ceramide], with the formation of a globoside-type of tetraglycosylceramide.[110] Transplantable, avian lymphoid tumours have been characterized by the absence of trisialogangliosides; moreover, the level of G_{M_2} is diminished and G_{M_1} is almost completely absent.[111] The net effect caused G_{M_3} and disialogangliosides to accumulate. A study of the kinetic properties of chicken-brain arylsulphatase A, using cerebroside 3-sulphate, has been reported.[112] A D-xylose-containing cerebroside has been isolated from the salt glands of herring gulls.[113] After intraocular application of 2-acetamido-2-deoxy-D-[^{3}H]mannose to fish, the sialic acid residues in the gangliosides were found to be more highly labelled than those in the glycoproteins.[114] Cycloheximide inhibited the incorporation of radioactivity into the gangliosides. The carbohydrate moiety of the globoside-type glycolipid from oysters was shown by g.l.c.–mass spectrometry to have L-fucose at the non-reducing terminus, and branch points at the 2 and 3 positions of D-galactose residues; D-glucose and *O*-alkylated L-fucose residues are also present.[115] The fragmentation pattern in electron-impact mass spectrometry of the permethylated methyl ester methyl glycoside of *N*-acetylneuraminic acid has been reported.[116] The mass spectral data have allowed a new sialic acid, isolated from the starfish *Distolasterias nipon*, to be identified as 8-*O*-methyl-*N*-acetylneuraminic acid. A purified sialoglycolipid containing two moles each of D-glucose and sialic acid has been obtained from gonads of the sea urchin *Strongylocentrotus intermedius*.[117] Residues of both *N*-acetyl- and *N*-glycolyl-neuraminic acids were shown to be present. Although periodate oxidation and methylation analysis did not differentiate between structures (21) and (22), the sum of the evidence clearly indicated that this is a new type of glycolipid.

Plant and Algal Glycolipids

A purified cerebroside from alfalfa leaves has been identified on the basis of i.r. spectroscopy, chemical analysis, and g.l.c. of the component sugar as *N*-hydroxydecanoyl-D-glucopyranosyldehydrophytosphingosine.[118] The phase behaviour in water of mono- and di-galactosyldiglycerides (from pelargonium leaves) and a sulpholipid (from a mixed algal colony) have been defined by *X*-ray diffraction methods.[119] The monogalactosyl-

[110] J. L. Chien, T. Williams, and S. Basu, *J. Biol. Chem.*, 1973, **248**, 1778.
[111] T. W. Keenan and R. L. Doak, *F.E.B.S. Letters*, 1973, **37**, 124.
[112] A. A. Faooqui and B. K. Bachhawat, *J. Neurochem.*, 1973, **20**, 889.
[113] K.-A. Karlson, B. E. Samuelsson, and G. O. Steen, *J. Lipid Res.*, 1972, **13**, 169.
[114] H. Rösner, H. Wiegandt, and H. Rahmann, *J. Neurochem.*, 1973, **21**, 655.
[115] T. Matsubara and A. Hayashi, *J. Biochem.* (*Japan*), 1973, **74**, 853.
[116] N. K. Kochetkov, O. S. Chizhov, V. I. Kadentsev, G. P. Smirnova, and I. G. Zhukova, *Carbohydrate Res.*, 1973, **27**, 5.
[117] N. K. Kochetkov, I. G. Zhukova, G. P. Smirnova, and I. S. Glukhoded, *Biochim. Biophys. Acta*, 1973, **326**, 74.
[118] S. Ito and Y. Fujino, *Canad. J. Biochem.*, 1973, **51**, 957.
[119] G. G. Shipley, J. P. Green, and B. W. Nichols, *Biochim. Biophys. Acta*, 1973, **311**, 531.

diglyceride formed a hexagonal lipid–water phase over an extended part of its phase diagram, and incorporation of water into the hexagonal lattice was limited to about 22 weight per cent. The rod-like structure of this phase was confirmed by freeze-etch electron microscopy, and dimensions calculated for the cylinder separation were in agreement with those revealed by *X*-ray measurements. The addition of a second D-galactose unit altered the phase behaviour; digalactosyldiglyceride, over an equivalent temperature-composition range, formed only a lamellar, lipid, bilayer phase, with limited uptake of water. Algal sulpholipids having a higher content of saturated fatty acids exhibited more complex behaviour: the lamellar phase appeared to exhibit limited swelling at low temperatures, whereas raising the temperature resulted in a gradual increase in inter-bilayer uptake of water.

The principal sugars detected in the acid lipids of cotton bolls included D-glucose and D-mannose, but significant proportions of L-arabinose and D-galactose were also present.[120] A particulate enzyme fraction from the cotton fibres catalysed the incorporation of D-mannose from GDP-D-[^{14}C]-mannose and D-glucose from UDP-D-[^{14}C]glucose into the acid lipids. Cycloheximide was shown to inhibit the biosynthesis of linolenic acid and galactolipids in the leaves of developing peas.[121] Brown rice grain has been shown to contain monogalactosyldiglyceride, digalactosyldiglyceride, and *N*-hydroxyarachidylglucosyl-2-sphingenine.[122] Column and thin-layer chromatography have been used to separate the mono- and di-glucosyl-glycerides of Italian millet (*Setarica italica*).[123] A more hydrophobic glycolipid than monogalactosyldiglyceride was isolated from *Spinacia oleracea*, and it was shown to contain residues of glycerol, D-galactose, and fatty acid (1 : 1 : 2).[124] From ^{1}H n.m.r. spectroscopy, mass spectrometry, and other data, it was considered to be a 1′-*O*-acyl-3′-*O*-(6-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol. Acylgalactosylmonoglyceride was postulated to originate *in vitro* from monogalactosylglyceride by accepting a fatty-acid residue from either digalactosyldiglyceride or another molecule of monogalactosylglyceride. Illumination of dark-grown seedlings of *Zea mays* produced an increase in the contents of mono- and di-galactosyl lipids, with two D-galactosyl residues donated by UDP-D-galactose.[125] Diolein was found to be an effective exogenous acceptor in the first galactosylation step, and endogenous maize monogalactosyldiglyceride performed a similar function in the second step.

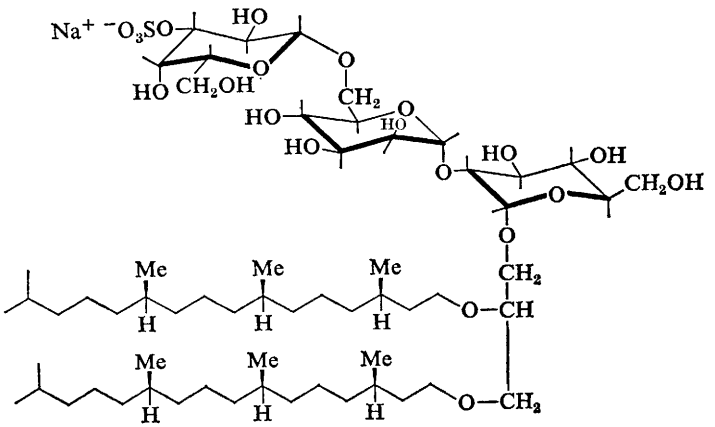
Four glycolipids have been isolated and purified from the envelope of the heterocyst of *Anabaena cylindrica*.[126] The position of the bond

[120] W. T. Forsee and A. D. Elbein, *J. Biol. Chem.*, 1973, **248**, 2858.
[121] J. Vichanska, A. Tremolières, and P. Mazliak, *Compt. rend.*, 1973, **277**, *D*, 501.
[122] Y. Fujino and S. Sakata, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2583.
[123] T. Obara and H. Kihara, *J. Agric. Chem. Soc. (Japan)*, 1973, **47**, 231.
[124] C. Critchley and E. Heinz, *Biochim. Biophys. Acta*, 1973, **326**, 184.
[125] B. N. Bowden, *Phytochemistry*, 1973, **12**, 1059.
[126] F. Lambein and C. P. Wolk, *Biochemistry*, 1973, **12**, 791.

between the sugar and the aglycone was determined by permethylation of the intact lipids, followed in sequence by hydrolysis, conversion of the methylated products into the TMS derivatives, and mass spectrometry. It was shown that C_{26} and C_{28} polyhydroxyalcohols are glycosylated at the terminal hydroxy-groups, and that C_{26} and, possibly, C_{28} hydroxy-fatty acids are glycosylated at the carboxylic acid groups.

Microbial Glycolipids

A glycolipid sulphate accounted for about 25% of the total polar lipids isolated from the extreme halophile *Halobacterium cutirubrum*.[127] D-Glucose, D-mannose, D-galactose, sulphate, and 2,3-di-*O*-phytanyl-*sn*-glycerol were released on vigorous acid hydrolysis of this glycolipid sulphate, whereas a galactosylmannosylglucosyldiphytanyl glycerol ether was liberated on mild hydrolysis. The positions of attachment of the sugars and the sulphate group were determined by methylation analysis, and the configurations of the glycosidic linkages were established by both optical data and hydrolyses with specific enzymes. The structure of the glycolipid sulphate was thereby established as 2′,3′-di-*O*-phytanyl-1′-*O*-[(β-D-galactopyranosyl 3-sulphate)-(1 → 6)-α-D-mannopyranosyl-(1 → 2)-α-D-glucopyranosyl]-*sn*-glycerol (23).



(23)

The growth medium and age of the culture affected the type and quantity of glycolipids produced by a halotolerant, moderately halophilic bacterium.[128] At high concentrations of salt in a nutrient broth, approximately equal amounts of glucosylphosphatidylglycerol and a D-glucuronic acid-containing glycolipid were obtained, whereas on replacement of the nutrient broth with one containing D-glucose as the sole source of carbon, the D-glucuronic acid-containing glycolipid accumulated, together with a

[127] M. Kates and P. W. Deroo, *J. Lipid Res.*, 1973, **14**, 438.
[128] N. Stern and A. Tietz, *Biochim. Biophys. Acta*, 1973, **296**, 130.

glucuronosyldiglyceride. The glucuronosyldiglyceride could also be synthesized from UDP-D-[^{14}C]glucuronic acid, added diglyceride, and a cell-free particulate fraction.[129] Periodate oxidation and digestion of a glucosylphosphatidylglycerol with an α-glucosidase suggested that phosphatidyl-2′-*O*-α-D-glucosylglycerol is the major product formed.[130] A toxic glycolipid resembling 'cord factor' (trehalose 6,6′-dimycolate) has been isolated from a virulent strain of *Mycobacterium tuberculosis*; from the content of trehalose and mycolic acid (1 : 1), it was indicated to be trehalose monomycolate.[131] Hydrogenolysis of peracetylated mycoside a′ resulted in cleavage between the aromatic nucleus and the acylated sugars.[132] Mass spectral investigation of a derivative of one of the sugars indicated that it is an ethyl 4-*O*-acetyl-6-deoxy-3-*O*-methyl-2-*O*-mycolylhexopyranoside. From the structures of mycosides A, B, and G, which consist of polar sugars bonded through an aromatic nucleus to an apolar aglycone, it was suggested that these substances could function as carriers or cofactors either in the vicinity of the bacterial membrane or close to the bacterial wall.

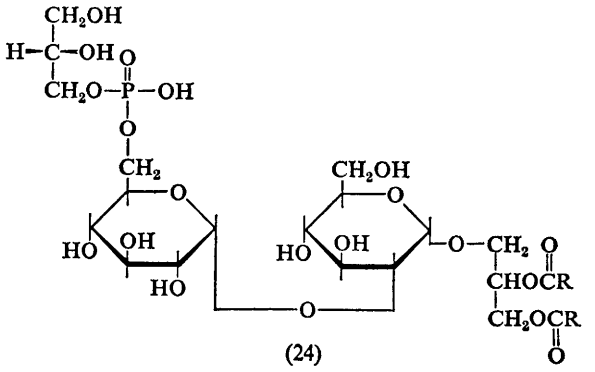
Binding of the glycolipid from *Salmonella minnesota* R-form to cultured, rat-embryo fibroblasts was demonstrated by the uptake of labelled glycolipid and by passive hemagglutination and immunofluorescence techniques.[133] Normal, rat-embryo fibroblasts exhibited a lower capacity for fixation than SV40-transformed, rat-embryo fibroblasts, with binding of the glycolipid localized at the cell surface. Based on the chemical alterations at the surface of normal cells following fixation of *S. minnesota* glycolipid by normal, transformed, and trypsinized cells, theoretical models for the insertion of glycolipid into the bilayer of normal and transformed cells were presented.[134] Binding of this glycolipid to the cell membrane of transformed cells elicited an increase in the intracellular level of cyclic AMP.[135]

The basic structure of the glycolipid from *Selenomonas ruminantium* has been identified as a 2-amino-2-deoxy-β-D-glucosyl-(1 → 6)-2-amino-2-deoxy-D-glucose unit to which *O*-acetyl groups and amide-linked fatty-acid residues are attached.[136]

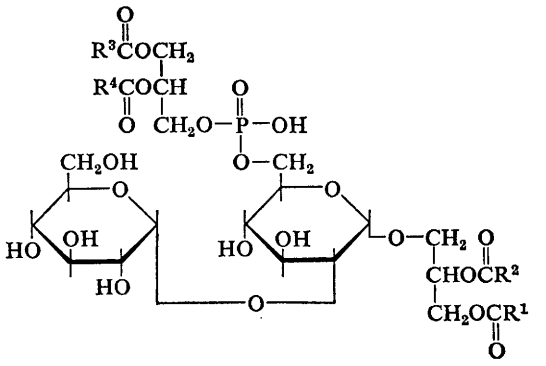
A C_{55} isoprenyl alcohol and its derivatives have been isolated from *Streptococcus faecalis*.[137] Three phosphoglycolipids, containing D-glucose, glycerol, fatty acid, and phosphate (2 : 2 : 2 : 1), have been isolated from

[129] N. Stern and A. Tietz, *Biochim. Biophys. Acta*, 1973, **296**, 136.
[130] E. Peleg and A. Tietz, *Biochim. Biophys. Acta*, 1973, **306**, 368.
[131] J. Maeda, *Jap. J. Bacteriol.*, 1972, **27**, 469.
[132] M. Gastambide-Odier, *European J. Biochem.*, 1973, **33**, 81.
[133] J. Bara, R. Lallier, C. Brailovsky, and V. N. Nigam, *European J. Biochem.*, 1973, **35**, 489.
[134] J. Bara, R. Lallier, M. Trudel, C. Brailovsky, and V. N. Nigam, *European J. Biochem.*, 1973, **35**, 495.
[135] G. Brailovsky, M. Trudel, R. Lallier, and V. N. Nigam, *J. Cell Biol.*, 1973, **57**, 124.
[136] Y. Kamio, K. C. Kim, and H. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2195.
[137] J. N. Umbreit, K. J. Stone, and J. L. Strominger, *J. Bacteriol.*, 1972, **112**, 1302.

Streptococcus faecalis and *S. hemolyticus*.[138] The structure of all three compounds was shown to be 1′(3′)-*O*-[6-(*sn*-glycerol 1′-phosphoryl)-*O*-α-D-glucopyranosyl-(1 → 2)-α-D-glucopyranosyl]diglyceride (24). The deacylated core was studied by periodate oxidation, Smith degradation,



(24)



(25)

alkaline hydrolysis, and enzymolysis. The fatty acids of phosphoglycolipids from *S. hemolyticus* were shown to be randomly distributed, whereas compounds from *S. faecalis* showed positional specificity, with preference for long-chain fatty acids at position 1 and short-chain fatty acids at position 2 of the glycerol moiety. A phosphatidyldiglucosyldiglyceride was also isolated from *S. faecalis*, and its relation to other polar lipids was investigated; it was shown to consist of D-glucose, glycerol, fatty acid, and phosphate (2 : 2 : 4 : 1).[139] Structural analysis of the deacylated core, based on periodate oxidation, Smith degradation,

[138] W. Fischer, I. Ischizuka, H. R. Landgraf, and J. Herrmann, *Biochim. Biophys. Acta*, 1973, **296**, 527.
[139] W. Fischer, H. R. Landgraf, and J. Herrmann, *Biochim. Biophys. Acta*, 1973, **306**, 353.

alkaline hydrolysis, and enzymic degradation, indicated the structure (25) for the glycolipid. A Streptococcus (defined as a Z_3 III strain) has been compared with a mutant strain (Z_3) lacking the type III polysaccharide antigen.[140] It was demonstrated that the membrane-free cell walls contain appreciable amounts of glycolipids, which were identified as mono- and di-glucosylglycerides. Parent bacteria and L-forms of a number of Streptococci of groups A and D and Streptococcus MG were shown to share common antigens.[141] Loss of serological reactivity between the Streptococci and the L-forms with either staphylococcal L-form or *Mycoplasma pneumoniae* occurred on removal of glycolipids.

Three glycolipids isolated from *Candida bogoriensis* were identified as 13-diglucosyloxydocosanoic acid and mono- and di-*O*-acetyl derivatives thereof.[142] Comparison of the mass spectra of these derivatives indicated that the acetyl group of the monoacetylated lipid is located on the internal D-glucose residue of β-D-glucopyranosyl-(1 → 2)-D-glucopyranosyloxydocosanoic acid. A study of the time course of production of the three glycolipids indicated that the diacylated derivative is the first extracellular product, and that the other forms result from deacylation. A glycolipid isolated from *Norcardia caviae* was shown to be an acid polyol glycosidically linked to D-glucose.[143] The sphingolipid from *Phycomycetes blakesleeanus* was found to contain D-glucose and D-galactose, whereas that from *Fusarium lini* contains only D-glucose.[144] Cell-free preparations from the protozoan *Tetrahymena pyriformis* incorporated D-glucose from UDP-D-[^{14}C]glucose into a chloroform-soluble glycolipid, which was stable to mild alkali, but labile to acid.[145] Indirect evidence suggested that the glycolipid is a glucosylphosphorylpolyprenol.

[140] J. H. J. Huis In't Veld and J. M. N. Willers, *J. Serol. Microbiol.*, 1973, **39**, 281.
[141] S. Birnbaum Feinman, B. Prescott, and R. M. Cole, *Infection and Immunity*, 1973, **8**, 752.
[142] T. W. Elders and R. J. Light, *J. Lipid Res.*, 1972, **13**, 663.
[143] M. T. Pommier and G. Michel, *Compt. rend.*, 1972, **275**, *C*, 1323.
[144] B. Weiss, R. L. Stilier, and R. C. M. Jack, *Lipids*, 1973, **8**, 25.
[145] R. W. Keenan, J. Matula, and L. Holloman, *Biochim. Biophys. Acta*, 1973, **326**, 84.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

BY J. F. KENNEDY

Recent advances in the chemistry, synthesis, analysis, modification, and hydrolysis of polysaccharides have been reviewed.[1]

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Enzymes, and Glycolipids

Polysaccharides.—The development of methods for the synthesis of polysaccharides from monosaccharides has been reviewed.[1]

Polymerization of 2,3,6-tri-*O*-(*N*-phenylcarbamyl)-D-glucopyranose by the action of phosphorus pentoxide in DMSO in the absence of catalysts and other solvents has been achieved.[2] The product of polymerization (DP 35) contained 0.3% phosphorus and was insoluble in either acetone or 1,4-dioxan, whereas the *N*-phenylcarbamyl derivatives of both amylose and cellulose were soluble in these solvents. Saponification (de-*N*-phenylcarbamylation) of the polymer with sodium methoxide in DMF–methanol furnished a product that was insoluble in water, dilute sodium hydroxide, DMF, DMSO, and pyridine, but that was soluble in 10% tetraethylammonium hydroxide. Although the i.r. spectrum did not permit the configuration of the inter-saccharide linkages to be assigned, the saponified polymer is considered to have a (1 → 4)-linked, cellulose-like structure, since no de-*N*-phenylcarbamylation occurred during polymerization, and acid hydrolysis yielded only D-glucose. The polymer was not degraded by either α- or β-amylase, whereas digestion with cellulase occurred to the extent of 65%. Rotational data for the saponified polymer and its peracetylated derivative, and ^{1}H n.m.r. spectral data for the latter derivative, confirmed that polymerization gave rise to a cellulose [β-(1 → 4)-linked D-glucan] type of polymer. It is considered that the bulky *N*-phenylcarbamyl groups prevent polymerization of the α-anomer.

A particulate enzyme preparation from *Phaseolus aureus* seedlings catalysed the synthesis of a water-insoluble β-(1 → 3)-glucan from UDP-α-D-glucopyranose at high concentration, whereas an alkali-insoluble,

[1] N. K. Kochetkov, in 'Chemistry of Natural Products – VIII', New Delhi, 1972, ed. T. R. Govindachari, International Union of Pure and Applied Chemistry, Butterworths, London, 1973, p.53.

[2] S. Hirano, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 187.

mixed β-(1 → 3 or 4)-glucan was obtained at low concentration.[3] Examination of the two reactions as a function of the concentration of UDP-α-D-glucopyranose revealed that the pH optimum, stability, activity, and metal-ion requirement of the enzyme differed in the two situations. Structural studies of the two types of polysaccharide produced were undertaken with the aid of *endo*-β-1,3-glucanase and cellulase.

Treatment of D-glucose with cold, concentrated sulphuric acid, followed by fractionation of the sodium salts of the products by dialysis, gave a non-dialysable product (16% yield) containing 19% sulphur.[4] The properties of this polymeric material were compared with those of other sulphated polysaccharides. The product was heterogeneous with respect to molecular size, and its properties were consistent with it being a mixture of polymers of sulphated-D-glucose residues. Titration data for the product accorded with the presence of monoester groups, and ^{1}H n.m.r. studies indicated that the polysaccharide chains are not branched. A moderately positive specific rotation was recorded for the polymer, suggesting the presence of a random mixture of α- and β-linkages.

Potato phosphorylase, immobilized either by covalent attachment (with the aid of glutaraldehyde or 2-amino-4,6-dichloro-*s*-triazine) to an alkylamine derivative of porous glass, or by adsorption on to colloidal silicic acid or poly(ethyleneimine)-coated silica, has been used in the synthesis of polysaccharides.[5] Each of the immobilized preparations synthesized polysaccharides, having a wide range of molecular weights, from D-glucose, the upper limit of which was governed by the solubility of the polysaccharides produced. The enzyme attached to porous glass was used in a continuous operation mode at 45 °C and possessed a half-life of 28 days under these conditions. The effect of primer [acid-degraded amylopectin of molecular weight 5×10^3] on the enzymic synthesis was examined, and the phosphorus contents, absorption maxima of the starch–iodine complexes, and viscosities of the products in water were determined. The polysaccharide was found to accumulate around the enzyme–glass particles, but could be removed by treatment with glucoamylase.

The development of a method for obtaining amylosucrase in a highly purified form from *Neisseria perflava* has permitted further studies of the catalytic properties of the enzyme. It was found that α-D-glucopyranosyl fluoride, like sucrose, is utilized both for glucosylating D-fructose (to form sucrose) and for the synthesis of a highly polymerized glycogen-like polysaccharide.[6] It was established that amylosucrase transfers an α-D-glucopyranosyl group, rather than an α-D-glucopyranosyloxy-group, and that the catalytic activity of the enzyme does not require the presence of pre-existing glycosidic linkages in the donor, but extends to the synthesis of glycosidic linkages where none existed previously. Using either sucrose

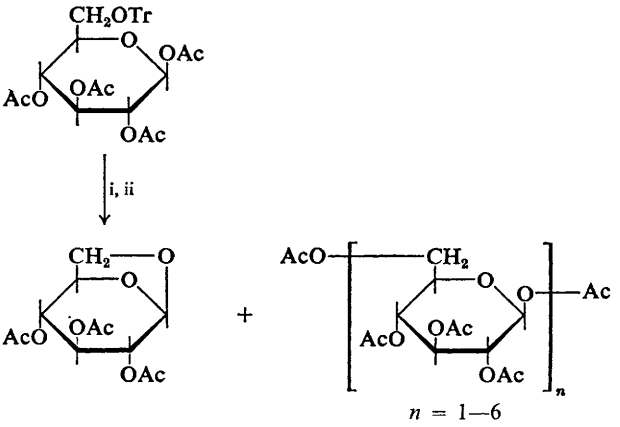
[3] C. Miyamoto and K. Tamari, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1253.
[4] K. Nagasawa and Y. Inoue, *Carbohydrate Res.*, 1973, **28**, 103.
[5] D. L. Marshall and J. Z. Walter, *Carbohydrate Res.*, 1972, **25**, 489.
[6] G. Okada and E. J. Hehre, *Carbohydrate Res.*, 1973, **26**, 240.

or α-D-glucopyranosyl fluoride, the enzyme synthesized a number of polysaccharides, which, on isolation, were found to give aqueous solutions that are more highly opalescent than those of mussel glycogen or potato amylopectin. The opalescence disappeared at once on addition of α-amylase. β-Amylase released maltose from both sets of products, which were also found to form soluble complexes with iodine in the same way as other α-D-glucans.

Two inducible enzymes, a levansucrase and a sucrase, have been shown to be responsible for the saccharolytic activity of *Bacillus subtilis*; on isolation in a pure form, the levansucrase was able to catalyse trans-fructosylation reactions to such acceptors as water, D-glucose, sucrose, and levans.[7] The apparent K_m values of the levansucrase for sucrose and levans of low molecular weight were determined, and a 90% yield of levan was obtained in the latter reaction. The enzyme also appears to occur in more than one strain of *B. subtilis*.

Oligosaccharides.—A review of the synthesis and determination of structure of oligosaccharides has appeared.[8] The methods of synthesis discussed include the uses of protected glycosyl halides, orthoesters, and oxazolines, *etc.*, for activation of the glycosidic centre. The review also deals with the synthesis of oligosaccharides from other oligosaccharides, the synthesis and properties of aglyconic components, and the determination of structure of synthetic oligosaccharides.

An extension of the use of fully acetylated sugars as glycosylating agents in the synthesis of oligosaccharides has been reported.[9] Thus, treatment of



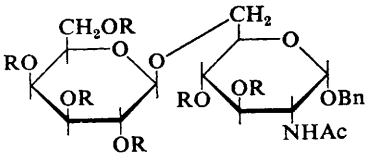
n = 1—6

Reagents: i, CH_2=$CHCH_2Br$–$AgClO_4$; ii, Ac_2O–AcONa

Scheme 1

[7] M. Pascal and R. Dedonder, *Carbohydrate Res.*, 1972, **24**, 365.
[8] N. K. Kochetkov, O. S. Chizhov, and A. F. Bochkov, in MTP International Review of Science, Series One, Vol. 7, 'Carbohydrates', ed. G. O. Aspinall, p. 147.
[9] V. A. Nesmeyanov, S. E. Zurabyan, and A. Y. Khorlin, *Tetrahedron Letters*, 1973, 3213.

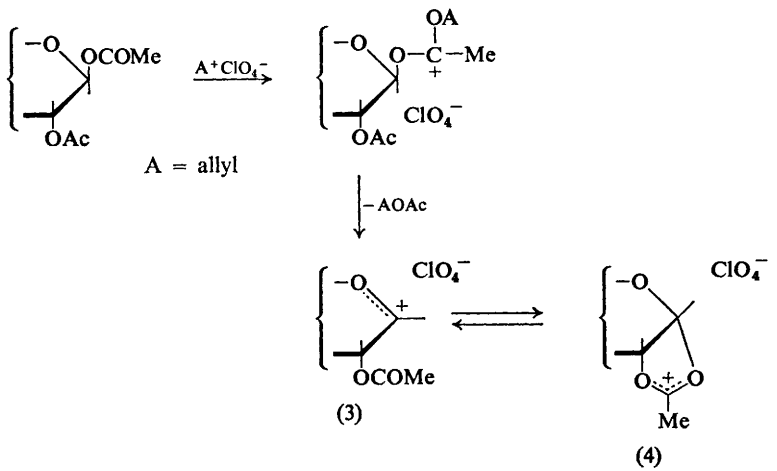
1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose with allyl bromide and silver perchlorate, followed by acetylation, gave rise to 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (Scheme 1), but analysis of the deacetylated products also showed the presence of D-glucose, gentiobiose, and higher oligosaccharides. Reaction of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose with benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside in benzene in the presence of allyl bromide and silver perchlorate gave benzyl 2-acetamido-3,4-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside (1) in low yield; an increased yield was obtained using nitromethane as the solvent. Deacetylation of disaccharide (1) gave benzyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (2), which on hydrogenolysis gave the known disaccharide 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-D-glucose, the structure of which was confirmed by its susceptibility to



(1) R = Ac
(2) R = H

β-glucosidase. The mechanism proposed for the reactions (Scheme 2) involves attack of the allyl cation on the sugar acetate to form the perchlorates (3) or (4), which are able to glycosylate the trityl ethers.

In addition to its hydrolase properties, an α-mannosidase from *Vicia sativa* seeds was able to transfer the D-mannosyl residue from phenyl

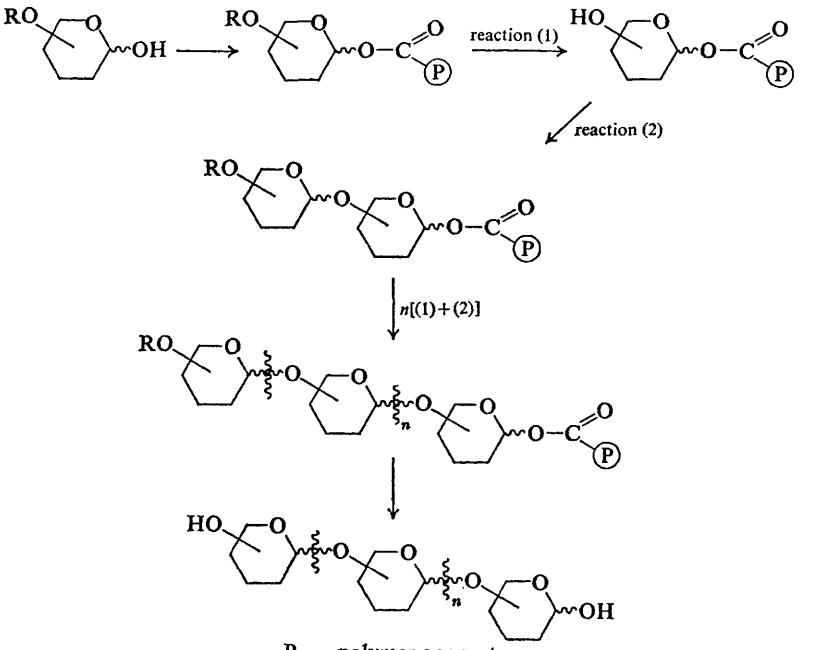


Scheme 2

α-D-mannopyranoside to ketoses and pentoses.[10] When D-ribose was used as the acceptor, three isomeric disaccharides were isolated and shown to be 2-, 3-, and 5-*O*-α-D-mannopyranosyl-D-ribose by methylation studies.

A purified β-xylosidase, extracted from a culture of *Aspergillus niger* on wheat-bran koji, was found to possess a glycosyltransferase activity.[11] The enzyme produced xylotriose from xylobiose, and xylotetraose and xylopentaose from xylotriose. Since the product oligosaccharides contain β-(1 → 4)-linkages, it appears that the enzyme preferentially transfers a D-xylosyl unit to C-4 of the appropriate xylo-oligosaccharides. This behaviour seems to be characteristic of the β-xylosidase of this organism, although it is well known that sugar residues are generally transferred to a number of positions on the acceptor sugars in the transglycosylation reactions of other glycosidases.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-[5-^{3}H]xylopyranose has been prepared from *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose by formation of the dimethyl dithioacetal, followed by treatment in sequence with periodate ion, sodium borotritide, and mineral acid.[12] *O*-(2-Acetamido-2-

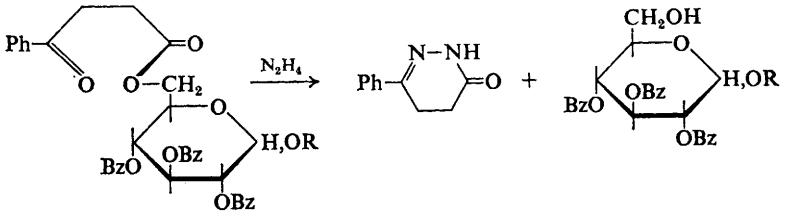


Scheme 3

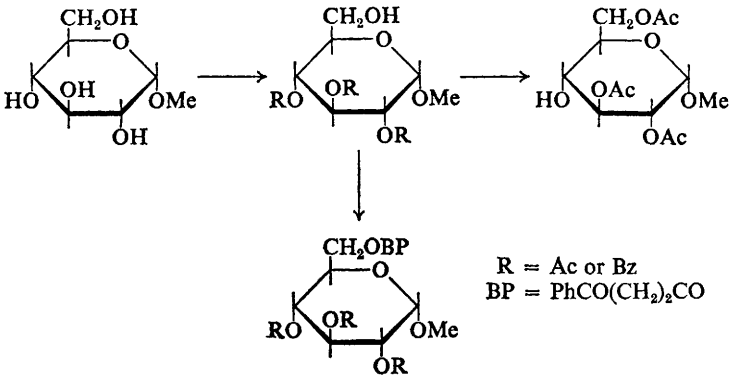
[10] A. Zurowska, E. Vilarroya, and F. Petek, *Carbohydrate Res.*, 1972, **24**, 319.
[11] S. Takenishi, Y. Tsujisaka, and J. Fukumoto, *J. Biochem. (Japan)*, 1973, **73**, 335.
[12] P. van Eikeren, W. A. White, and D. M. Chipman, *J. Org. Chem.*, 1973, **38**, 1831.

deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-L-[5-^{3}H]arabinopyranose was also isolated as a by-product from the reaction. The D-xylose-containing disaccharide was also isolated from the lysozyme-catalysed reaction of [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)]$_3$-2-acetamido-2-deoxy-D-glucopyranose with 2-acetamido-2-deoxy-α-D-[5-^{3}H]xylopyranose, thereby confirming the structure of the disaccharide obtained by chemical synthesis. β-(1 → 4)-Linkages were assumed for the higher oligosaccharides, containing 2-acetamido-2-deoxy-D-xylose and either two or three 2-acetamido-2-deoxy-D-glucose residues, also obtained as products of the enzymic synthesis.

Various methods for the synthesis of oligosaccharides on solid-phase, water-insoluble supports have been reported. In one paper, possible syntheses of oligosaccharides on polymeric supports were discussed briefly, and the reactions adopted in principle (Scheme 3) involved the selective removal, in the presence of other ester groups, of a temporary blocking group.[13] With this in mind, the β-benzoylpropionyl group was studied; and it was found that, when substituted at C-6 of a D-glucoside, this group could be selectively removed with hydrazine if the other hydroxy-groups are benzoylated (Scheme 4), but that its removal is accompanied by side-reactions if the other hydroxy-groups are acetylated. An acetyl



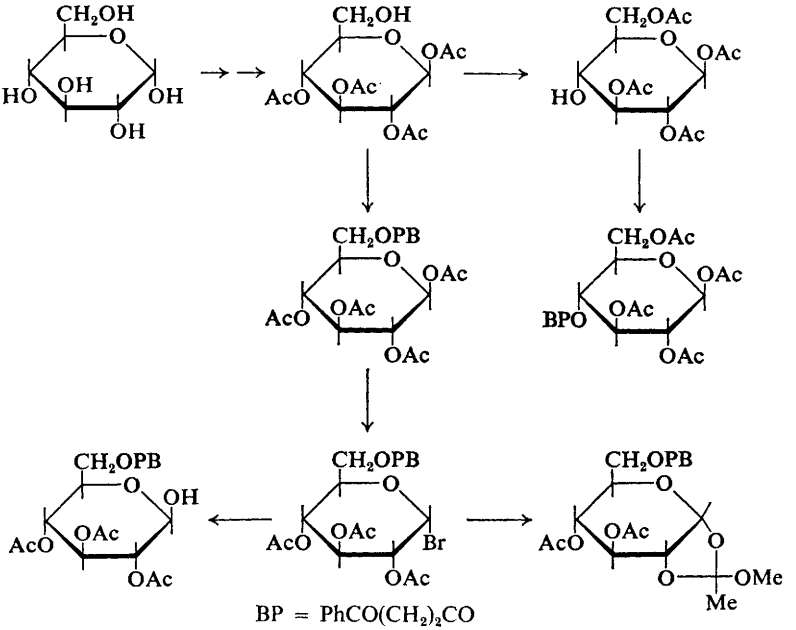
Scheme 4



Scheme 5

[13] N. Belorizky, G. Excoffier, D. Gagnaire, J.-P. Utille, M. Vignon, and P. Vottero, *Bull. Soc. chim. France*, 1972, 4749.

group at C-1, however, could be liberated without affecting the β-benzoylpropionyl group substituted at C-6. Furthermore, the 6-(β-benzoylpropionyl) group was stable to conditions used for the formation of oligosaccharides using Koenigs–Knorr or orthoester-type reactions. In the light of these findings, syntheses of a number of D-glucose derivatives carrying the β-benzoylpropionyl group were reported (Schemes 5 and 6).

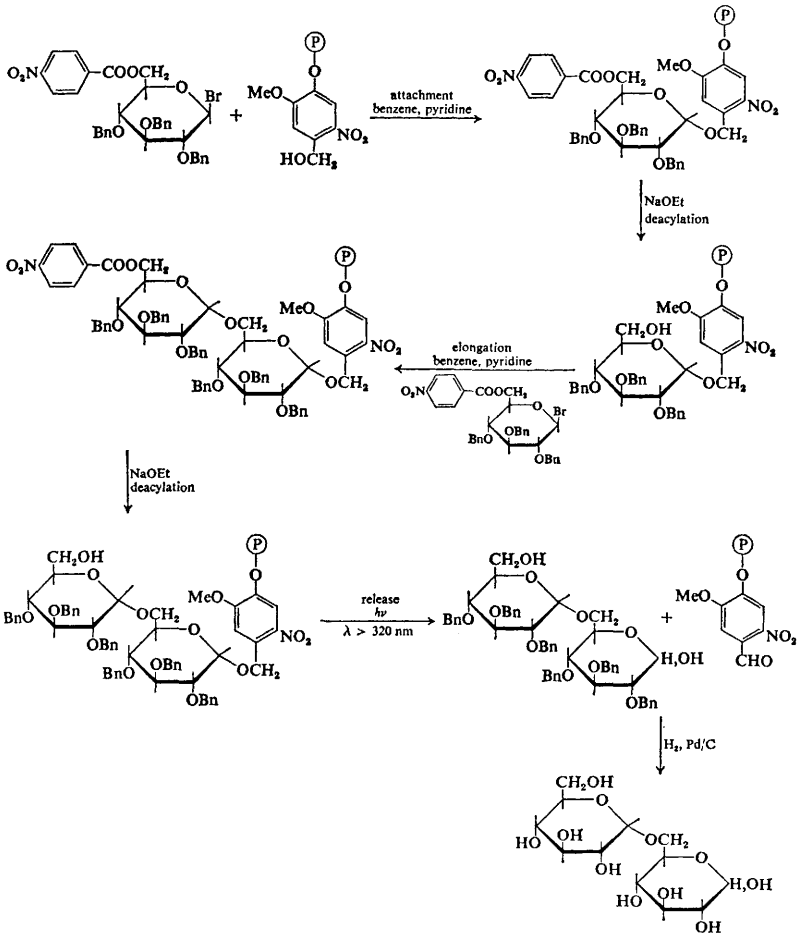


Scheme 6

As a support for the solid-phase synthesis of oligosaccharides, a light-sensitive polymer has been synthesized by attaching 6-nitrovanillin, *via* an ether linkage, to a copolymer of chloromethylated styrene and divinylbenzene.[14] The aldehydic functions on the polymer were reduced with sodium borohydride to alcohol groups, which were used for subsequent condensation reactions (see Scheme 7). Irradiation of the polymer suspended in 1,4-dioxan released the carbohydrate moiety, leaving the resin in the aldehydic form. The soluble products were debenzylated and identified as D-glucose and isomaltose.

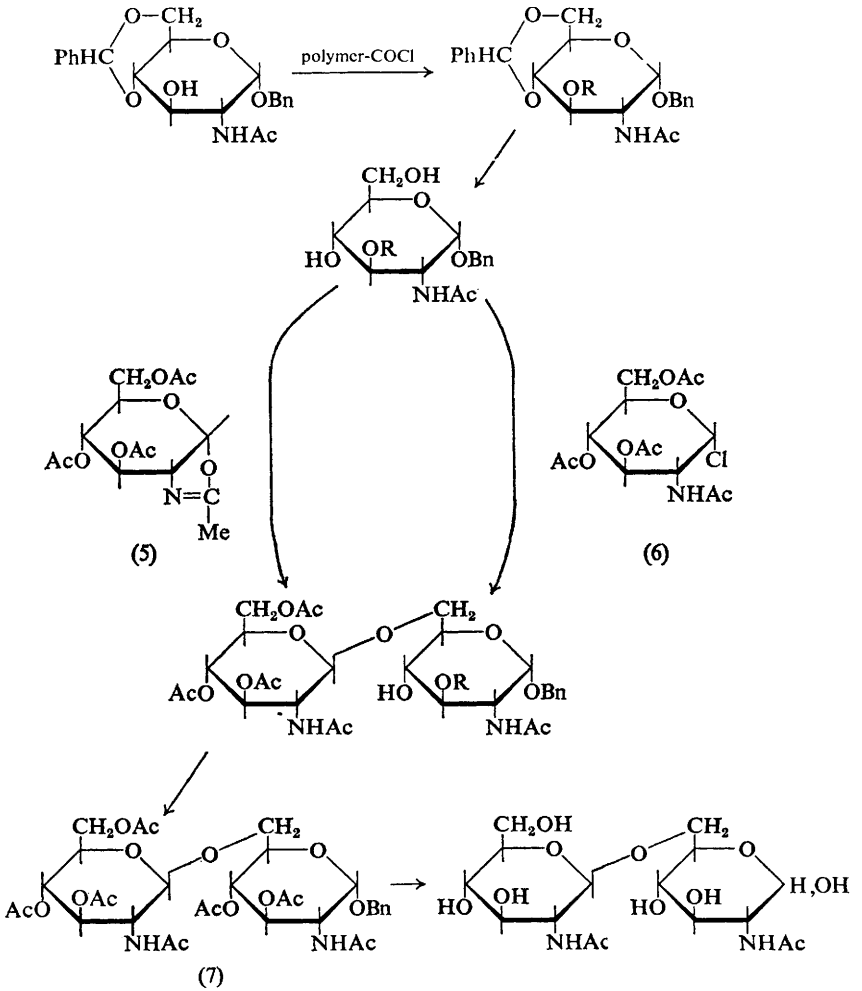
A solid-phase procedure can be used for the synthesis of oligosaccharides containing 2-acetamido-2-deoxyhexose units.[15] A cross-linked, styrene–divinylbenzene 'popcorn' polymer, having acid chloride functional groups, was used as the matrix, which was treated in a mixture of benzene and

[14] U. Zehavi and A. Patchornik, *J. Amer. Chem. Soc.*, 1973, **95**, 5673.
[15] G. Excoffier, D. Gagnaire, J.-P. Utille, and M. Vignon, *Tetrahedron Letters*, 1972, 5065.



Scheme 7

pyridine with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (Scheme 8). After esterification of the residual acid chloride groups with methanol and removal of the benzylidene group with aqueous trifluoroacetic acid, glycosylation was effected with either 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-2-oxazoline (5) or 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (6). The product was presumed to be the polymer-fixed, protected, β-(1 → 6)-linked disaccharide, on the basis of the favoured stereochemistry of the glycosylation reactions of (5) and (6), and the higher reactivity of the primary hydroxy-group. Three successive treatments were necessary to achieve 80% coupling. Detachment of the protected disaccharide from the resin was effected

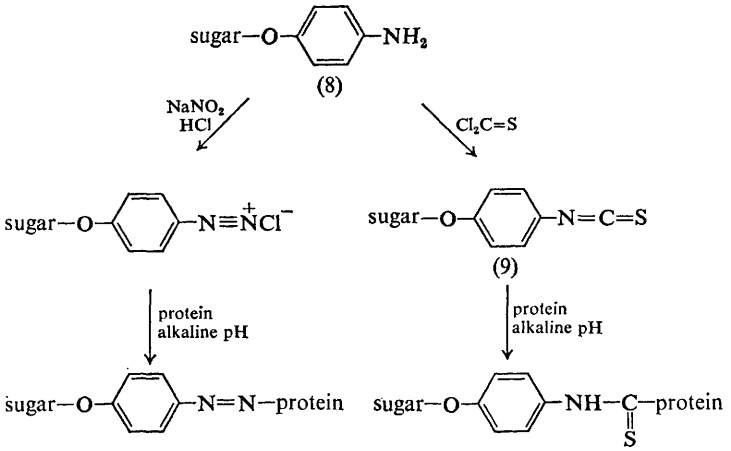


Scheme 8

either with methanol–toluene saturated with ammonia or, more effectively, with sodium methoxide in methanol–1,4-dioxan. Acetylation of the detached product yielded benzyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside (7), which was identified by mass spectrometry and i.r. spectroscopy. Another product formed in the coupling reaction appeared to be a trisaccharide derivative, presumably arising from glycosylation at both O-4 and O-6. Deacetylation of (7) with methanolic ammonia, followed by hydrogeno-

lysis, gave 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose.

Glycoproteins.—Various methods for the coupling of carbohydrates to proteins to form carbohydrate antigens (*pseudo*-glycoproteins) have been described in detail. Reactions requiring an alkaline pH were the conjugation of *p*-aminophenyl glycosides (8) and phenylisothiocyanato-glycosides (9) derived therefrom with protein (Scheme 9) – reactions that have been



Scheme 9

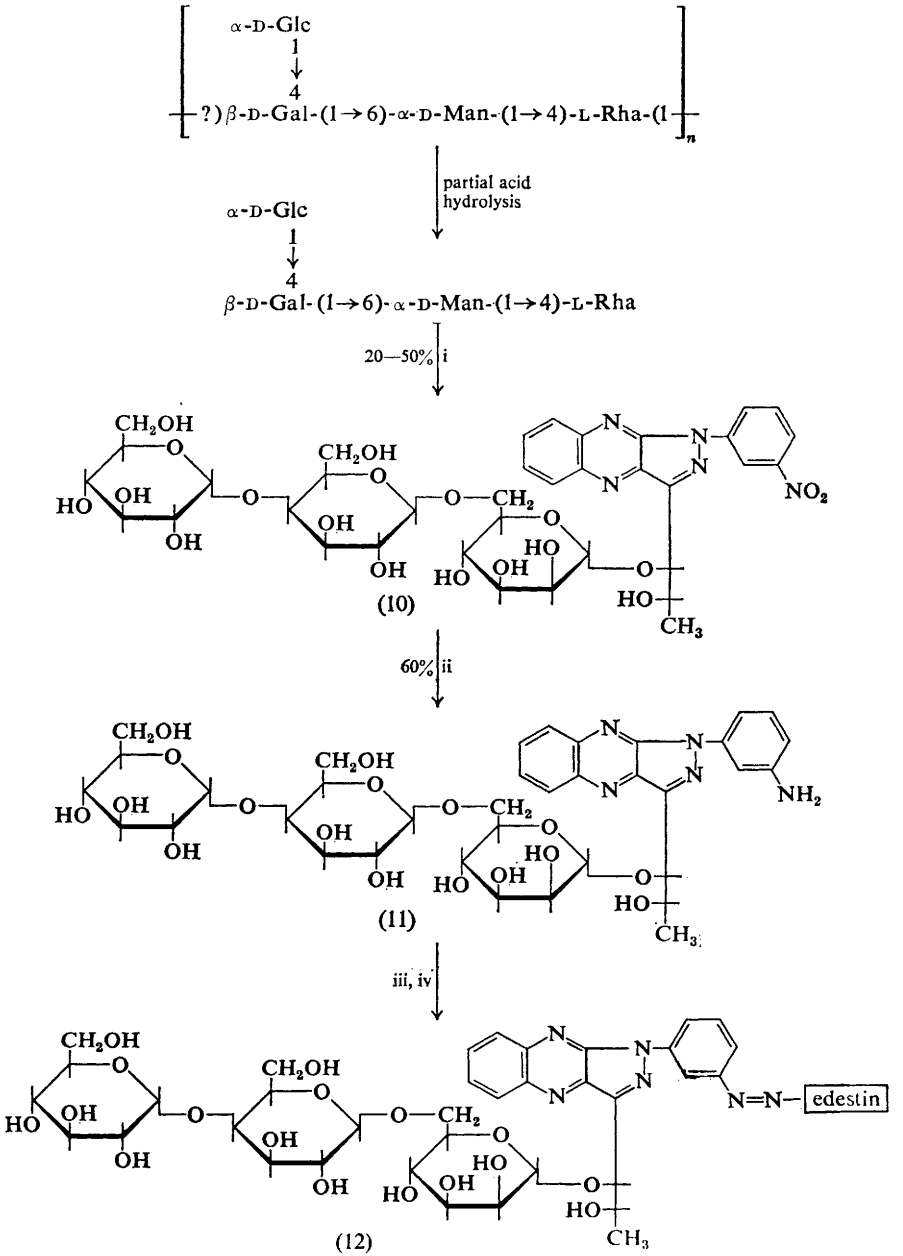
applied to many proteins.[16] An alternative method used isomaltose oligosaccharides that had been oxidized at the reducing terminals to the corresponding aldonic acids.[17] These acidic oligosaccharides can be coupled to the amino-groups of proteins with the aid of isobutyl chloroformate in aqueous DMF.

The reaction of reducing oligosaccharides with 3-nitrophenylhydrazine and 1,2-diaminobenzene has been shown to yield the 1-(3-nitrophenyl)-flavazole derivative [*e.g.* (10)], which can be hydrogenated to the corresponding 1-(3-aminophenyl)flavazole (11).[18] Diazotization of the latter and reaction with a protein yielded a conjugate in which the carbohydrate and protein are covalently linked; the conjugate may be used as a carbohydrate antigen. The reaction sequence was illustrated by the preparation of a flavazole–azoedestin conjugate (12) from the tetrasaccharide repeating

[16] C. R. McBroom, C. H. Samanen, and I. J. Goldstein, in 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Vol. XXVIII, ed. V. Ginsburg, Academic Press, New York, 1972, p. 212.

[17] G. Ashwell, in 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Vol. XXVIII, ed. V. Ginsburg, Academic Press, New York, 1972, p. 219.

[18] K. Himmelspach and G. Kleinhammer, in 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Vol. XXVIII, ed. V. Ginsburg, Academic Press, New York, 1972, p. 222.



Reagents: i, H^+–$H_2NNHC_6H_4NO_2$-*m*–$H_2NC_6H_4NH_2$-*o*; ii, H_2–$Pd/BaSO_4$; iii, diazotization; iv, edestin

Scheme 10

unit of the O-specific side-chain of the lipopolysaccharide of *Salmonella illinois* (Scheme 10).

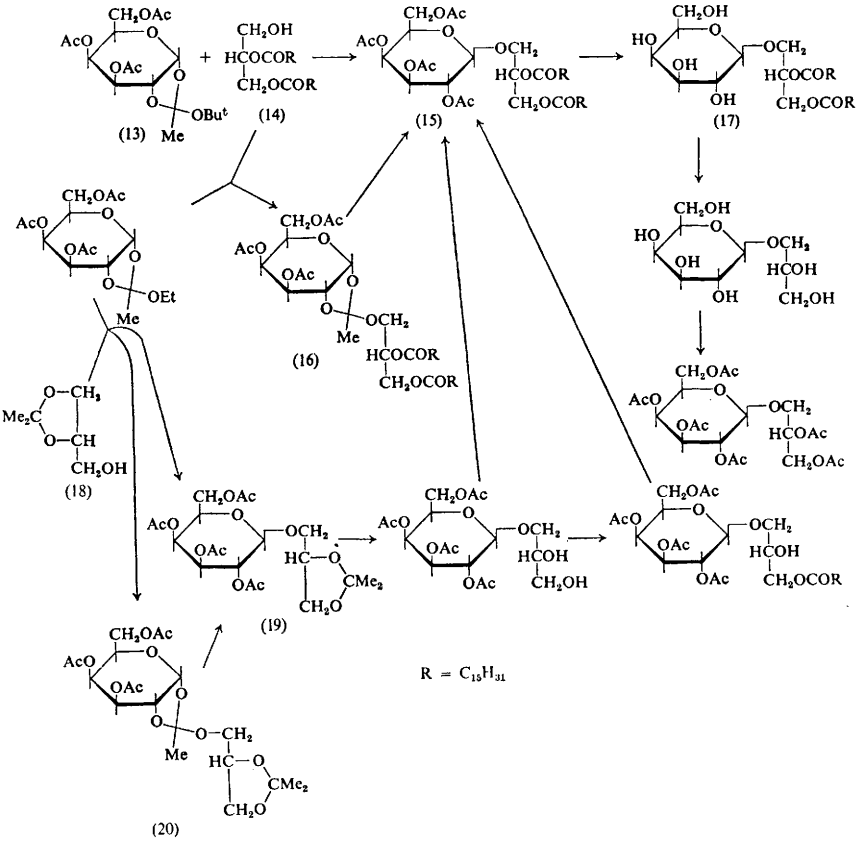
Attempts have been made to prepare immunogenic molecules by the reaction of δ-*N*-(2,4-dinitrophenyl)-L-ornithine hydrochloride with a cyclic imidocarbonate derivative of Sepharose, and also by the reaction of Bio-Gel (cross-linked polyacrylamide in the hydrazide form) with 2,4-dinitrobenzenesulphonate.[19] Assessment of the immunogenicity of the gels showed that only the Sepharose-gel derivative is active, eliciting the generation of large numbers of splenic anti-2,4-dinitrophenyl plaque-forming cells.

Enzymes.—The results of two Merrifield (solid-phase) syntheses of hen egg-white lysozyme have been published.[20] An improved procedure yielded a product having the correct molecular weight (by gel filtration) and a non-reducible form of high molecular weight. Only the former possessed enzymic activity, which, for the crude preparation, was 0.5—1.0% of that of the native enzyme. Following chromatography on chitin, the specific activity was raised to 2—3% of that of the native form or to 9—25% of that of a native form that had been subjected to the same experimental conditions as the synthetic product.

Gangliosides.—Tritium-labelled gangliosides, $G_{T1\ a/b}$, G_{D1a}, G_{Db1}, G_{M1}, and G_{M2} (Tay–Sachs ganglioside), have been prepared *in vivo* using a tritium-labelled 2-acetamido-2-deoxy-D-mannose as precursor.[21] The resulting polysialogangliosides were converted into labelled ganglioside G_{M1} by the action of neuraminidase, and this ganglioside was then transformed into the Tay–Sachs ganglioside by the action of β-galactosidase. Examination of the tritium distribution of labelled G_{M2} showed that all the tritium atoms are located in the *N*-acetylneuraminic acid residues, but the 2-acetamido-2-deoxy-D-galactose residues are not labelled. Analogous preparations of gangliosides $G_{T1\ a/b}$, G_{D1a}, G_{D1b}, and G_{M2}, labelled with tritium in the *N*-acetylneuraminic acid residues using 2-[^{3}H]acetamido-2-deoxy-D-mannose, have been reported; the separate gangliosides were produced.[22]

Glycolipids.—Syntheses of D-galactosylglycerides possessing the structure of naturally occurring glycerides, using the orthoester method of glycosylation, have been reported (Scheme 11).[23] 3,4,6-Tri-*O*-acetyl-1,2-*O*-(t-butylorthoacetyl)-α-D-galactopyranose (13) reacted with 1,2-di-*O*-palmitoyl-*sn*-glycerol (14) to yield 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2-di-*O*-palmitoyl-*sn*-glycerol (15), which was also obtained by way of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1,2-di-*O*-palmitoyl-*sn*-glyceryl-3-*O*-orthoacetyl)-β-D-galactopyranose (16). Deacetylation of the glyceryl glycoside (15) yielded 3-*O*-β-D-galactopyranosyl-1,2-di-*O*-palmitoyl-*sn*-glycerol (17), modi-

[19] G. N. Trump, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 544.
[20] J. J. Sharp, A. B. Robinson, and M. D. Kamen, *J. Amer. Chem. Soc.*, 1973, **95**, 6097.
[21] E. H. Kolodny, R. O. Brady, J. M. Quirk, and J. N. Kanfer, *J. Lipid Res.*, 1970, **11**, 144.
[22] J. F. Tallman and R. O. Brady, *Biochim. Biophys. Acta*, 1973, **293**, 434.
[23] A. I. Bashkatova, G. V. Smirnova, V. N. Volynskaya, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1973, **9**, 1393.

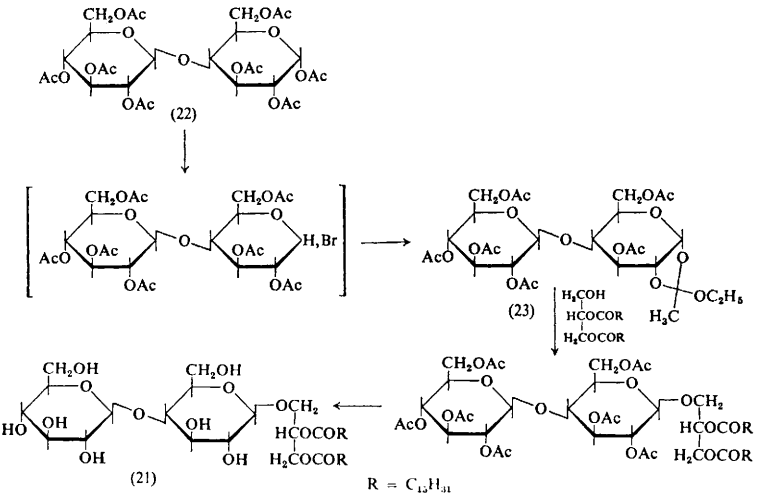


Scheme 11

fied forms of which were also produced. Using the orthoester (13) and 1,2-*O*-isopropylidene-*sn*-glycerol (18), it was also possible to form 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-*sn*-glycerol (19) by way of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1,2-*O*-isopropylidene-*sn*-glyceryl-3-orthoacetyl)-α-D-galactopyranose (20). De-blocking of the product (19), followed by esterification, yielded (15), from which the natural D-galactosyldiglyceride (17) was obtained. The orthoester (20) was also prepared by the reaction of 1,2-*O*-isopropylidene-*sn*-glycerol with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide.

The synthesis of 3-*O*-(4-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-1,2-di-*O*-palmitoyl-*sn*-glycerol (21) from cellobiose octa-acetate (22) is outlined in Scheme 12; glycosylation was achieved using cellobiose ethyl orthoacetate hexa-acetate (23).[24]

[24] A. I. Bashkatova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1972, **8**, 2277.



Scheme 12

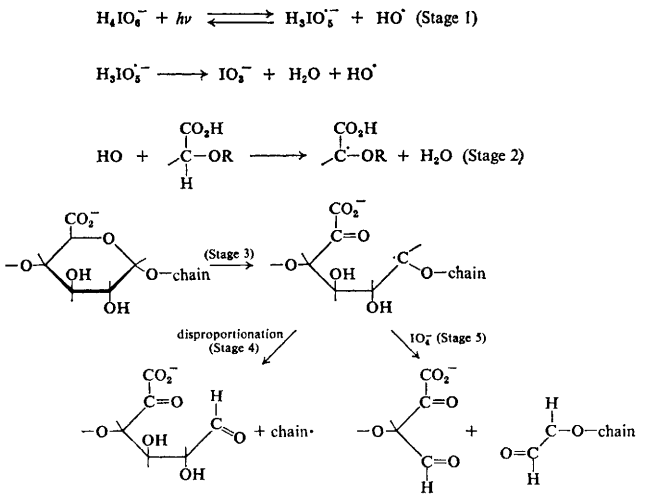
Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

Recent developments in the chemistry of polysaccharides have been reviewed.[1]

Periodate-induced decreases in the viscosity of aqueous solutions of acetal- and ether-linked polymers (in the case of polysaccharides oxidation leads to dialdehyde derivatives) have been compared with the action of periodate on polymers that are neither polyethers nor polyacetals.[25] A mechanism for the degradation of polysaccharides was proposed based on a known disproportionation of ether-type free-radicals induced by hydroxy-radicals in periodate solution. Scission of the polymer chain could occur solely by ether-type disproportionation, or by glycol cleavage following ring opening caused by disproportionation involving the ring-oxygen atom. The susceptibility of glycuronans to periodate oxidation is considered to be due, in part, to the ease of formation of free radicals by abstraction of H-5, followed by ring opening and glycol fission by periodate (Scheme 13).

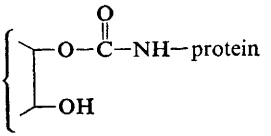
The nature of the linkage formed when compounds containing amino-groups react with cyclic imidocarbonate derivatives of polysaccharides has been investigated further.[26] The approach taken was to evaluate (by isoelectric focusing) any changes in the net charge resulting from modification of subtilisin with cyclic imidocarbonate derivatives of malto-oligosaccharides. Conversion of lysyl residues into *N*-alkylcarbamates (24) is likely to result in a loss of positive charge on the protein surface in the pH

[25] J. E. Scott and M. J. Tigwell, *Carbohydrate Res.*, 1973, **28**, 53.
[26] B. Svenson, *F.E.B.S. Letters*, 1973, **29**, 167.

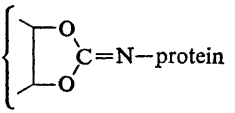


Scheme 13

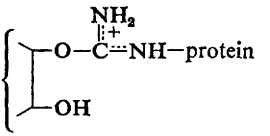
range 0—10. Since the pK_a of a model N-substituted imidocarbonate, ethyl isobutyl imidocarbonate, was found to be 4.7, it was assumed that imidocarbonates are much weaker bases than protein amino-groups; thus, formation of an N-substituted imidocarbonate (25) would cause a decrease in the number of protonated groups on the protein in the pH range 6—10. However, the formation of an N-substituted isourea (26) was considered to be without influence on the charge on the protein, since the dissociation constants of isoureas and protein amino-groups are of the same order of mag-



(24)



(25)



(26)

nitude. Isoelectric focusing of the subtilisin derivative indicated that isoureas are major products of the reaction of protein lysyl groups with polysaccharide cyclic imidocarbonates (see below p. 539).

The i.r. spectra of a number of polysaccharides, which had been nitrated in various ways, have been studied.[27] The range 960—1350 cm^{-1} was found to be useful for distinguishing the nitrated molecules compared with the range 730—960 cm^{-1} for the identification of the polysaccharides themselves. The high intensity of the nitrate bands limited the interpretation of spectra of nitrated polysaccharides below 960 cm^{-1}, but above this frequency the absorption bands are generally sharper and more clearly defined than the corresponding bands of the parent polysaccharide. Data for the C—O—C (bridge), C—C (ring), C—O, and C—OH frequencies, and for the C—H deformation and stretching frequencies were reported, and the use of i.r. spectroscopy for the quantitative determination of nitrate groups in nitrated polysaccharides was discussed.

Derivatives of polysaccharides have continued to be used as matrices for the covalent immobilization of enzymes, and a further review on immobilized enzymes has appeared.[28] Derivatives of polysaccharides have also continued to be used extensively as matrices for the production of affinity-chromatography materials, and the affinity chromatography of enzymes [29] and various macromolecules [30] has been reviewed.

Methods reported for the visualization of proteins bound to polysaccharide matrices have involved either pre-derivatization of the protein to be coupled with fluorescein isothiocyanate, or treatment of the coupled protein with its homologous antibody that had been allowed to react with fluorescein isothiocyanate.[31]

Agar.—An α_1-glycoprotein from human serum has been found to exhibit a high affinity for agar, with which it formed a complex.[32]

Agarose.—Commercially available, macroporous agarose in bead forms (*e.g.* Sepharose and Bio-Rad Agarose) has been converted into cyclic imidocarbonate derivatives by reaction with cyanogen bromide; these derivatives have continued to be used extensively in the immobilization of biologically active macromolecules and for affinity chromatography. The reaction of the derivatized agarose gel with the incoming molecule is considered to occur by nucleophilic attack of amino-groups on the carbon atom of the *trans*-imidocarbonate ring to give either an isourea or an N-substituted imidocarbonate or an N-substituted carbonate (see also pp. 509 and 539). Recent references to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes), active immobilized forms of immunologically active macromolecules (for use as

[27] A. F. Dawoud and A. Marawan, *Carbohydrate Res.*, 1972, **26**, 65.
[28] K. L. Smiley and G. W. Strandberg, *Adv. Appl. Microbiol.*, 1972, **15**, 13.
[29] I. A. Cherkasov, *Uspekhi Khim.*, 1972, **41**, 1911.
[30] P. Cuatrecasas, *Adv. Enzymol.*,1972, **36**, 29.
[31] J. Lasch, M. Iwig, and H. Hanson, *European J. Biochem.*, 1972, **27**, 431.
[32] H. Haupt, N. Heimburger, T. Kranz, and S. Baudner, *Z. physiol. Chem.*, 1972, **353**, 1841.

immunoadsorbents), and various affinant molecules (for use as affinity-chromatography matrices) by such methods are summarized in Tables 1,[33–37] 2,[38–41] and 3,[42–76] respectively. The derivatives are listed according to the prescribed uses, since, for example, the insoluble form of an enzyme used for affinity chromatography may not have been enzymically active.

Using insulin B-chain as an example, it has been demonstrated that the site of attachment of the molecule to agarose cyclic imidocarbonate may be varied by carrying out the coupling under different conditions.[68] Thus, it

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme	*E.C. No.*	*Ref.*
Aminopeptidase (cytosol) labelled with fluorescein isothiocyanate	3.4.11.1	31
β-Amylase	3.2.1.2	33 [a, b]
Chymotrypsin	3.4.21.1	34 [a]
Fructose-bisphosphate aldolase	4.1.2.13	35
Polyribonucleotide nucleotidyltransferase	2.7.7.8	36
Ribonuclease I	3.1.4.22	37 [a]

[a] Using beads of agarose cross-linked with epichlorohydrin before conversion into imidocarbonate derivatives; [b] See (35) and (35B) (isocyanide route), p. 516.

Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Aminopeptidase (cytosol)	Determination of protein binding to agarose cyclic imidocarbonate using anti-enzyme labelled with fluorescein isothiocyanate	31
Anti-2,4-dinitrophenyl	Isolation of trypsin; by complexation of a crude enzyme preparation with 2,4-dinitrophenylated soybean trypsin inhibitor, affinity chromatography of the complex, and cleavage of the eluted complex	38
Anti-μ light-chains of immunoglobulins	Removal of immunoglobulin IgM from normal human serum	39
Immunoglobulin IgG	Removal of antibodies to light chains of immunoglobulins from an antiserum containing antibodies to both light- and μ-chains	39

[33] P. Vretblad and R. Axén, *Biotechnol. Bioeng.*, 1973, **15**, 783.
[34] J. Porath, K. Aspberg, H. Drevin, and R. Axén, *J. Chromatog.*, 1973, **86**, 53.
[35] J. S. Mort, D. K. K. Chong, and W. W. C. Chain, *Analyt. Biochem.*, 1973, **52**, 162.
[36] J. C. Smith, I. J. Stratford, D. W. Hutchinson, and H. J. Brentnall, *F.E.B.S. Letters*, 1972, **30**, 246.
[37] R. Axén, J. Carlsson, J. C. Janson, and J. Porath, *Enzymologia*, 1971, **41**, 359.
[38] M. Wilchek and M. Gorecki, *F.E.B.S. Letters*, 1973, **31**, 149.
[39] J. D. Sipe and F. V. Schaefer, *Appl. Microbiol.*, 1973, **25**, 880.

Table 2 (*cont.*)

Immunologically active component coupled to agarose cylcic imidocarbonate	*Use of product*	*Ref.*
Rabbit immunoglobulin IgG	Isolation of anti-rabbit immunoglobulin IgG	40
Rabbit immunoglobulin IgG anti-deoxyribonuclease	Isolation of deoxyribonuclease	41
Sheep immunoglobulin IgG anti-deoxyribonuclease	Isolation of deoxyribonuclease	41
Whole human-serum proteins	Removal of antibodies to normal human-serum proteins from anti-hepatitis sera	39
	Removal of light-chain antibodies from anti-human immunoglobulin IgM sera	39

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials*

Ligand or affinant	*Use of product*	*Ref.*
2-Acetamido-*N*-(ε-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine	Purification of wheat-germ agglutinin	42
3-Aminobenzamidine	Purification of thrombin and trypsin	43
4-Aminobenzamidine	Purification of trypsin	43
ε-Aminocaproylglycyl-leucine	Isolation of neutral protease from *Clostridium histolyticum*	44
ε-Aminocaproyl-leucine amide	Isolation of neutral protease from *Clostridium histolyticum*	44
ε-Aminocaproyl-D-tryptophan methyl ester	Isolation of stem bromelain	45
	Hydrophobic affinity chromatography of proteins and glycoproteins (including enzymes and immunoglobulins)	46
6-Amino-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside	Purification of galactosyltransferase	47
	Purification of wheat-germ agglutinin	48
P′-(6-Amino-1-hexyl)-P²-(β-D-galactopyranosyl) pyrophosphate	Purification of galactosyltransferase	47
P′-(6-Amino-1-hexyl)-P²-(5′-uridine) pyrophosphate	Purification of galactosyltransferase	47

[40] D. Catty, J. F. Kennedy, R. L. Drew, and H. Cho Tun, *J. Immunol. Methods*, 1973, **2**, 353.
[41] K. W. Ryder and M. E. Hodes, *J. Chromatog.*, 1972, **80**, 128.
[42] R. Lotan, A. E. S. Gussin, H. Lis, and N. Sharon, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 656.
[43] H. F. Hixon and A. H. Nishikawa, *Arch. Biochem. Biophys.*, 1973, **154**, 501.
[44] L. G. Sparrow and A. B. McQuade, *Biochim. Biophys. Acta*, 1973, **302**, 90.
[45] D. Bobb, *Preparative Biochem.*, 1972, **2**, 347.
[46] B. H. J. Hofstee, *Analyt. Biochem.*, 1973, **52**, 430.
[47] R. Barker, K. W. Olsen, J. H. Shaper, and R. L. Hill, *J. Biol. Chem.*, 1972, **247**, 7135.
[48] J. H. Shaper, R. Barker, and R. L. Hill, *Analyt. Biochem.*, 1973, **53**, 564.

Table 3 (*cont.*)

Ligand or affinant	*Use of product*	*Ref.*
3-Aminophenyl β-D-gluco-pyranuronoside	Purification of β-glucuronidase	49
4-Aminophenylguanidine	Purification of trypsin	43
Colchicine (a mixture of deacetyl and isodeacetyl derivatives)	Isolation of brain tubulin	50
Concanavalin A	Isolation of glycogen (starch) synthetases	51
	Purification of α_1-antitrypsin	52
	Purification of α-fetoprotein	53
	Purification of other phytohaem-agglutinins	54
	Purification of serum α_1-antitrypsin	55
	Separation by affinity chromatography, analysis, and identification of mono-, oligo-, and poly-saccharides	56, 57
Deoxyribonucleic acid (single-stranded and heat-denatured)	Affinity chromatography of deoxyribonucleases	58
2′-Deoxyuridine-5′-(6-*p*-nitrobenzamido)hexyl phosphate in hydrogenated form	Purification of thymidylate synthetase	59
1,2-Diaminoethane 1,4-Diaminobutane 1,6-Diaminohexane	Isolation of tRNA synthetases	60
δ-*N*-2,4-Dinitrophenyl-L-ornithine hydrochloride	Immunogen for eliciting a large number of splenic anti-2,4-dinitrophenyl plaque-forming cells	19
Fetuin	Affinity chromatography of cholera toxins of *Vibrio cholerae*	61
Folate-binding protein	Isolation and removal of folates from goat milk	62
Gentamicin	Purification of an acetyltransferase involved in the inactivation of gentamicin	63
Glutathione (oxidized to dimer; disulphide linkage)	Purification of glutathione reductase [NAD(P)H]	64

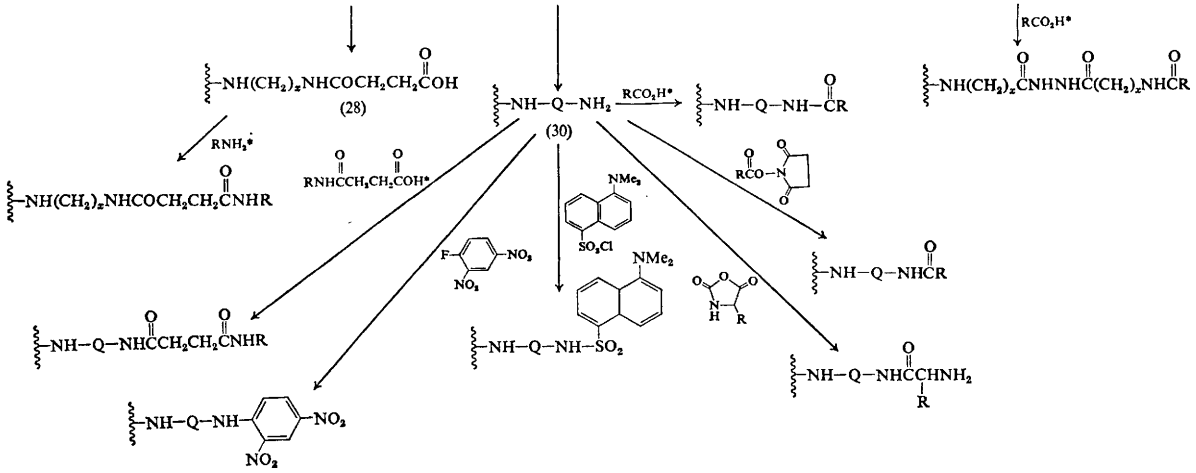
[49] R. G. Harris, J. J. M. Rowe, P. S. Stewart, and D. C. Williams, *F.E.B.S. Letters*, 1973, **29**, 189.
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[55] I. E. Liener, O. R. Garrison, and Z. Pravda, *Biochem. Biophys. Res. Comm.*, 1973, **51**, 436.
[56] J. F. Kennedy and A. Rosevear, *J.C.S. Perkin I*, 1973, 2041.
[57] J. F. Kennedy, *L'Actualité Chimique*, 1973, No. 4, p. 67.
[58] J. C. Schabort, *J. Chromatog.*, 1972, **73**, 253.
[59] P. V. Danenberg, R. J. Langenbach, and C. Heidelberger, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1029.
[60] H. Jakubowski and J. Pawełkiewicz, *F.E.B.S. Letters*, 1973, **34**, 150.
[61] P. Cuatrecasas, I. Parikh, and M. D. Hollenberg, *Biochemistry*, 1973, **12**, 4253.
[62] J. Selhub and N. Grossowicz, *F.E.B.S. Letters*, 1973, **35**, 76.
[63] F. Le Goffic and N. Moreau, *F.E.B.S. Letters*, 1973, **29**, 289.
[64] J. J. Harding, *J. Chromatog.*, 1973, **77**, 191.

Table 3 (*cont.*)

Ligand or affinant	*Use of product*	*Ref.*
Glycine	Affinity chromatography of threonine dehydratase	65
Glycyl-leucine	Isolation of neutral protease from *Clostridium histolyticum*	44
Glycyltyrosine	Purification of sialidase	66
Haptoglobin	Affinity chromatography of haemoglobin and removal of free haemoglobin from haemolysed sera	67
Insulin	Purification of insulin-specific protease	68
Isoleucine	Affinity chromatography of threonine dehydratase	65
Kanamycin A	Purification of an acetyltransferase involved in the inactivation of gentamicin	63
α-Lactalbumin	Purification of *N*-acetyl-lactosamine synthetase	69
Leucine	Affinity chromatography of threonine dehydratase	65
Leucine amide	Isolation of neutral protease from *Clostridium histolyticum*	44
Nicotinamide adenine dinucleotide	Purification of glyceraldehyde phosphate dehydrogenase	70
Ovo-inhibitor (chicken)	Isolation of alkaline proteases from *Aspergillus oryzae*	71
4-Phenylbutylamine	Hydrophobic affinity chromatography of proteins and glycoproteins (including enzymes and immunoglobulins)	46
Phytohaemagglutinin from *Lens culinaris*	Purification of virus glycoproteins	72
Poly(deoxyribonucleotide)	Affinity chromatography of deoxyribonucleases	58
Poly(DL-lysine)	Isolation of T4 phage	73
Pregnancy-serum proteins	Assay of plasma testosterone	74
Proteoglycan glycopeptide linkage glycopeptide [a]	Purification of β-acetamidodeoxyhexosidase	75
t-Ribonucleic acid	Purification of aminoacyl ribonucleic acid synthetase	76

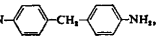
[a] See p. 554.

[65] I. Rahimi-Laridjawi, H. Grimminger, and F. Lingens, *F.E.B.S. Letters*, 1973, **30**, 185.
[66] J. I. Rood and R. G. Wilkinson, *Proc. Austral. Biochem. Soc.*, 1973, **6**, 9.
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[71] G. Feinstein and A. Gertler, *Biochim. Biophys. Acta*, 1973, **309**, 196.
[72] M. I. Hayman, J. J. Skeael, and M. J. Crumpton, *F.E.B.S. Letters*, 1973, **29**, 185.
[73] L. Sundberg and S. Höglund, *F.E.B.S. Letters*, 1973, **37**, 70.
[74] H.-U. Fisch, V. Pliška, G. I. Tesser, R. Schwyzer, and M. Zachmann, *F.E.B.S. Letters*, 1973, **29**, 127.
[75] G. Dawson, R. L. Propper, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1102.
[76] H. Hayashi, *J. Biochem.* (*Japan*), 1973, **74**, 203.



R = rest of affinant, ligand, glycoprotein, enzyme, etc.;
Q = rest of proteinaceous bridge molecule;
* = reaction involving carbodi-imide.

Scheme 14†

† In subsequent references to this scheme, the suffixes A, B, and C denote the use of $H_2N(CH_2)_xNH(CH_2)_xNH_2$, H_2N-  -NH_2, or H_2N--NH_2, respectively, in place of $H_2N(CH_2)_xNH_2$ for the production of (27).

was possible to couple selectively the insulin B-chain by either the ε-amino-group of lysine-29 or the amino-group of the terminal phenylalanine residue.

A method reported for the visualization of matrix-bound proteins involved the reaction of aminopeptidase (cytosol) with fluorescein isothiocyanate prior to coupling with agarose cyclic imidocarbonate.[31] Alternatively, the enzyme was immobilized in the normal way using the agarose derivative, and the bound enzyme was located by treatment with its homologous antibody that had been labelled with fluorescein isothiocyanate.

A method for determining the amount of glycoproteinaceous material coupled to agarose cyclic imidocarbonate has been reported.[77] The method is based on determination of the amount of glycoproteinaceous carbohydrate bound, and was exemplified using a number of glycopeptides derived from glycoproteins.

Direct comparison of the immunoadsorbent capacities of an immunoglobulin IgG covalently attached either to an agarose cyclic imidocarbonate or to cellulose *trans*-2,3-cyclic carbonate showed that the latter possesses a number of superior qualities.[40]

Chromatographic studies have been reported of the affinities of a number of purified proteinaceous macromolecules for agarose (macroporous beads) cyclic imidocarbonate treated with either 4-phenylbutylamine or ε-aminocaproyl-D-tryptophan methyl ester.[46] The affinities were compared with those of untreated agarose and agarose cyclic imidocarbonate. At pH 8, chymotrypsin and immunoglobulin bound irreversibly to the 4-phenylbutylamine derivative; whereas serum albumin, β-lactoglobulin, and ovalbumin bound strongly to this derivative, they could be eluted to different extents by concentrated aqueous solutions of salts. In the latter cases, elution by the salt was enhanced by the addition of a polarity-reducing agent such as ethylene glycol. Chymotrypsin and the immunoglobulin also exhibited high affinities for the D-tryptophanyl derivative. The findings suggested that the binding of proteinaceous molecules to the agarose derivative results from the combined and, possibly, mutually reinforcing effects of hydrophobic and electrostatic forces. An application of the hydrophobic affinity was demonstrated by chromatography of a mixture of proteins on a column of the 4-phenylbutylamine–agarose derivative. Several estero proteolytic enzymes in crude preparations were separated from the bulk of the protein and from each other by chromatography on the D-tryptophan–agarose derivative.

In a number of instances, direct attachment of affinity ligands to the polysaccharide derivative proved to be unsatisfactory for affinity chromatography, since the approach of the incoming molecule to the ligand is sterically inhibited. This problem has been overcome by incorporating a bridge between the matrix and the ligand. The bridging method has also been used when functional group(s) on the ligand are incompatible with

[77] G. Sepulchre and E. Moczar, *J. Chromatog.*, 1973, **75**, 114.

Table 4 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials* via *linkage extension*

Ligand or affinant	*Agarose intermediate* [a]	*Use of product*	*Ref.*
1-*O*-(2-Acetamido-2-deoxy-β-D-gluco-pyranosyl)-6-amino-hexan-1-ol	(28)	Purification of *N*-acetyl-lactosamine synthetase	69
6-Aminopenicillanic acid	(28A)	Isolation of penicillin-binding components from membranes of *Bacillus subtilis* cells	78
4-Aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside	(28A)	Separation and purification of β-galacto-sidase, β-glucuronidase, and β-acet-amidodeoxyglucosidase	79
4-Aminophenyl β-D-galactopyranoside	(28A)	Separation and purification of β-galacto-sidase, β-glucuronidase, and β-acet-amidodeoxyhexosidase	79
4-Aminophenyl β-D-glucopyranuronoside	(28A)	Separation and purification of β-galacto-sidase, β-glucuronidase, and β-acet-amidodeoxyhexosidase	79
4-Aminophenyl melibioside	(28A)	Affinity chromatography of α-galacto-sidases	80, 81
4-Aminophenyl 1-thio-β-D-galacto-pyranoside	(28A)	Purification of β-galactosidase Isolation of β-galactosidases by single-step affinity chromatography	82
N-(4-Diazophenyl)oxamic acid	(29)	Purification of sialidase	66
Oestradiol-17β-monohemisuccinate	(27)	Specific adsorption of α-fetoprotein and the isolation of anti-α-fetoprotein	83

[78] P. M. Blumberg and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3751.
[79] E. Junowicz and J. E. Paris, *Biochim. Biophys. Acta*, 1973, **321**, 234.
[80] C. A. Mapes and C. C. Sweeley, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 1317.
[81] C. A. Mapes and C. C. Sweeley, *J. Biol. Chem.*, 1973, **248**, 2461.
[82] P. J. Robinson, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1972, **285**, 28.
[83] R. Arnon, E. Teicher, M. Bustin, and M. Sela, *F.E.B.S. Letters*, 1973, **32**, 335.

Table 4 (*cont.*)

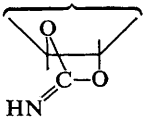
Ligand or affinant	*Agarose intermediate* [a]	*Use of product*	*Ref.*
α-Fetoprotein [b]	Oestradiol-17β-monohemisuccinate on (27)	Isolation of anti-α-fetoprotein	83
D-Galactono-1,4-lactone	(27C)	Purification of α- and β-galactosidases	84
Gangliosides [c]	(27A) (30; Q = albumin) [30; Q = branched multichain copolymer of poly(L-lysine) backbone and DL-alanine side-chains] (30; Q = urea-denatured albumin) (30; Q = fetuin) [30; Q = poly(L-lysine)] (30; Q = thyroglobulin)	Isolation of cholera toxin from *Vibrio cholerae*	61
Glutathione (oxidized to dimer, disulphide bridge)	(27), (31)	Purification of glutathione reductase [NAD(P)H]	64
N-Hydroxysuccinimide esters of gangliosides	(27A) (30; Q = albumin) [30; Q = branched multichain copolymer of poly(L-lysine) backbone and DL-alanine side-chains] (30; Q = urea-denatured albumin) (30; Q = fetuin) [30; Q = poly(L-lysine)] (30; Q = thyroglobulin)	Isolation of cholera toxin from *Vibrio cholerae*	61
N-Hydroxysuccinyl pepstatin	(27)	Purification of renin	85

Nicotinamide adenine dinucleotide	(32)	Purification of glyceraldehyde phosphate dehydrogenase	86
L-Phenylalanine	(33)	Isolation of phenylalanine:tRNA ligase	87
Phloretin	(32A)	Purification of D-glucose-binding protein	88
Poly(DL-alanine *N*-carboxyanhydride)	[30; Q = poly(D- or L-lysine)]	Affinity chromatography of various materials	89
Potassium oxalate	(27)	Affinity chromatography and separation of lactate dehydrogenase isoenzymes	90
Saccharo-1,4-lactone	(27), (27A)	Purification of β-glucuronidase	49
N^6-Succinyl adenosine monophosphate	[30; Q = poly(D- or L-lysine)]	Affinity chromatography of various materials	89
Various ligands	[30; Q = poly(D- or L-lysine)] [30; Q = poly(DL-alanine)-poly(D- or L-lysine)]	Affinity chromatography of various materials	89

[a] See Scheme 14; [b] Adsorbed on to agarose intermediate; [c] Utilizing the carboxy-groups of *N*-acetylneuraminic acid units for coupling.

[84] J. N. Kanfer, G. Petrovich, and R. A. Mumford, *Analyt. Biochem.*, 1973, **55**, 301.
[85] P. Corvol, C. Devaux, and J. Menard, *F.E.B.S. Letters*, 1973, **34**, 189.
[86] J. D. Hocking and J. I. Harris, *F.E.B.S. Letters*, 1973, **34**, 280.
[87] P. I. Forrester and R. L. Hancock, *Canad. J. Biochem.*, 1973, **51**, 231.
[88] F. F. Fannin and D. F. Diedrich, *Arch. Biochem. Biophys.*, 1973, **158**, 919.
[89] M. Wilchek, *F.E.B.S. Letters*, 1973, **33**, 70.
[90] H. Spielmann, R. P. Erickson, and C. J. Epstein, *F.E.B.S. Letters*, 1973, **35**, 19.

those on the matrix, since a new type of group can be introduced by means of an appropriate bridging molecule. Reaction sequences recently applied to agarose cyclic imidocarbonates for the production of affinity-chromatography matrices, *etc.*, are depicted in Scheme 14, and the final derivatives so obtained for affinity chromatography are summarized in Table 4,[78–90] and were all derived from structure (36). A colorimetric test for the conversion



(36)

of the agarose cyclic imidocarbonate into the succinyl derivative (28A) has involved reaction of the derivative with 2,4,6-trinitrobenzenesulphonate.[78] When the bridge was provided by a molecule already attached to the matrix, it was found that beads modified by coupling an amine to agarose cyclic imidocarbonate did not readily dissolve in hot water, presumably owing to the formation of cross-linkages during the activation step or, in the case of diamino-compounds, during the initial treatment.[91] Studies on modified agaroses (27), (27A), (28), and (34A) showed that warming in solutions of urea or guanidinium chloride did not render the materials soluble, whereas warming in acid or alkali did; for example, all four derivatives dissolved in either 0.1M hydrochloric acid or M sodium hydroxide at 75 °C. With the exception of (34A), the derivatives were also soluble in 50% acetic acid at the same temperature. Once dilute solutions were formed (~10% v/v), the pH could be adjusted to neutrality without visible precipitation of the solute. The dilute solutions were stable and transparent in the u.v. and visible ranges, depending on the method of solubilization. In general, covalently bound ligands possessing chromophoric groups, or functional groups that could be converted into chromophores, could be quantitated by direct spectrometric analysis, provided a suitable solubilizing medium was chosen. Specific derivatives determined in this way were picrylsulphonic acid coupled to (27) and (27A) (solubilized with acetic acid), the *N*-hydrosuccinimide ester of *S*-carboxymethyl-21-thioprogesterone coupled to (27), and 3-iodotyrosine coupled to agarose cyclic imidocarbonate (solubilized by alkali containing 0.1% borohydride). In the case of a bridge provided by the reaction of agarose cyclic imidocarbonate with poly(D- or L-alanine), the number of amino-groups remaining for attachment of a ligand was ascertained either by reaction with 2,4-dinitrofluorobenzene, followed by estimation of ε-2,4-nitrophenyllysine after total hydrolysis, or by reaction with dansyl chloride.[89] Both the dansyl and 2,4-dinitrophenyl derivatives of poly(D- or L-alanine)-agarose were stable to physiological conditions, indicating a satisfactory stability of the poly(amino-acid) bridge.

[91] D. Failla and D. V. Santi, *Analyt. Biochem.*, 1973, **52**, 363.

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	46
1-Aminobutane, Aminoethane, 2-Aminoethanol, 1-Aminopropane	(28A)	Experimental control for affinity chromatography trials	79
Bovine fibrinogen glycopeptide, Calf corneal glycoprotein glycopeptide	—	Demonstration of coupling of glycopeptides to agarose imidocarbonate, and for assay of degree of coupling by measurement of coupled carbohydrate	77
Concanavalin A	—	Investigation of the mitogenic activity of the agarose–concanavalin A complex on spleen cells	92
Dansyl chloride	[30; Q = poly(D- or L-lysine)]	Determination of amino-groups available in the intermediate by hydrolysis and estimation of ε-dansyl-lysine	89
Desialylated bovine fibrinogen glycopeptide	—	Demonstration of coupling of glycopeptides to agarose cyclic imidocarbonate, and for assay of the degree of coupling by measurement of coupled carbohydrate	77
2,4-Dinitrofluorobenzene	[30; Q = poly(D- or L-lysine)]	Determination of amino-groups available in the intermediate by hydrolysis and estimation of ε-2,4-dinitrophenyl-lysine	89
F-Actin	—	Studies *in vitro* of the biological activity of F-actin filament	93
Glycyl-leucine	—	Determination of the capacity of the agarose derivative	34, 34 [b]

[92] M. Ono, H. Maruta, and D. Mizuno, *J. Biochem.* (*Japan*), 1973, **73**, 235.
[93] H. Kondo, H. Hayashi, and K. Mihashi, *J. Biochem.* (*Japan*), 1972, **72**, 759.

Table 5 (*cont.*)

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Glycyltyrosine	(29)	Experimental control for affinity chromatography experiments	66
Human fibrinogen glycopeptide	—	Demonstration of coupling of glycopeptides to agarose cyclic imidocarbonate, and for assay of the degree of coupling by measurement of coupled carbohydrate	77
N-Hydroxysuccinimide ester of *S*-carboxymethyl 21-thioprogesterone	(27)	Demonstration of methods for the quantitation of ligands attached to agarose cyclic imidocarbonate	91
3-Iodotyrosine	—		
Picrylsulphonic acid	(27), (27A)		
N-Terminal-acetylated bovine-fibrinogen glycopeptide	—	Demonstration of coupling of glycopeptides to agarose cyclic imidocarbonate, and for assay of the degree of coupling by measurement of coupled carbohydrate	77

[a] See Scheme 14; [b] Agarose beads cross-linked with epichlorohydrin before conversion into the cyclic imidocarbonate.

Miscellaneous products obtained by coupling various molecules to agarose cyclic imidocarbonates are listed in Table 5.

By performing the activation of agarose beads with cyanogen bromide in alkaline phosphate solutions of very high buffer capacity, the control of pH that has hitherto been necessary can be omitted.[34] Strongly,ly moderately, and weakly activated gels could be prepared simply and reproducibly in this way. The capacities of the gels were determined by coupling with glycyl-leucine, and the ability to immobilize enzymes with retention of activity was demonstrated using chymotrypsin. Parallel experiments with bead forms of agarose gels cross-linked with epichlorohydrin were equally successful.

5′-(4-Aminophenyl)uridine 2′,3′-cyclic phosphate (a strongly competitive inhibitor of ribonuclease) has been attached to agarose, and the derivative was used in purifying the enzyme by affinity chromatography.[94]

Commercially prepared beads of a mixture of agarose and polyacrylamide have been treated with glutaraldehyde and ampicillin to give a material that was used for the affinity chromatography of β-lactamase.[95]

Cross-linked forms of agarose have been prepared by treating the macroporous material with epichlorohydrin.[33, 34, 37] The cross-linked materials were converted into the corresponding cyclic imidocarbonates by the action of cyanogen bromide, and the products were used for immobilization of chymotrypsin [34] and ribonuclease I [37] (by direct attachment) and β-amylase (by the isocyanide route) [see (35) and (35B) (Scheme 14) and Table 1]. The capacity of the imidocarbonate derivative of cross-linked agarose was determined using glycyl-leucine.[34]

An agarose-type matrix has been used in the formation of immobilized forms of enzymes.[96] Agarose was mixed with persulphate and *NNN′N′*-tetramethylethylenediamine to which was added a mixture of acrylamide, methylenebisacrylamide, and the enzyme that had been cross-linked with glutaraldehyde.

An alkaline solution of single-stranded deoxyribonucleic acid in solidified agarose has been used in the separation of bacterial ribonucleases.[97] Since haemagglutinins from the seeds of *Sophora japonica* were found to associate with agarose, macroporous agarose in bead form has been employed in their purification by affinity chromatography.[98]

Alginic Acid and Alginates.—The rapid decrease in the viscosity of solutions of alginate in the presence of periodate ion has been compared with the behaviours of other polysaccharides (containing carboxy-groups) and

[94] R. K. Wierenga, J. D. Huizinga, W. Gaastra, G. W. Welling, and J. J. Beintema, *F.E.B.S. Letters*, 1973, **31**, 181.
[95] F. Le Goffic, R. Labia, and J. Andrillon, *Biochim. Biophys. Acta*, 1973, **315**, 439.
[96] G. Broun, D. Thomas, G. Gellf, D. Domurado, A. M. Berjonneau, and C. Guillon, *Biotechnol. Bioeng.*, 1973, **15**, 359.
[97] S. C. Weatherford, L. S. Weisberg, D. T. Achord, and D. Apirion, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1307.
[98] T. Terao and T. Osawa, *J. Biochem. (Japan)*, 1973, **74**, 199.

polymers under the same conditions.[25] A mechanism for the cleavage of alginate was suggested, based on the radical reactions shown in Scheme 13.

Whereas the presence of carboxy-groups prevented the formation of *cis*-2,3-cyclic carbonate groups on alginic acid on treatment with ethyl chloroformate, under conditions that converted cellulose into the *trans*-2,3-cyclic carbonate, derivatization of the corresponding propylene glycol ester was successful.[99] The presence of *cis*-2,3-cyclic carbonate groups, and also of a number of acyclic carbonate (ethyloxycarbonyl) groups, in the product was deduced from i.r. spectroscopy. From similar reactions with other polysaccharides and cycloamyloses, it appears that the relative proportion of cyclic and acyclic groups introduced is related to the reaction conditions rather than to the availability of free hydroxy-groups at C-6. Applications of the polysaccharide derivative in the field of immobilization were discussed.

Treatment of hydroxypropylalginate with sodium hydroxide has confirmed that depolymerization is much slower and more dependent on the concentration of alkali than the analogous depolymerization of pectin.[100] The unsaturated products of depolymerization were determined by the uptake of bromine, and evidence was obtained for their formation by an *E*1cb mechanism. It was concluded that there is a facile β-elimination in polymers of (1 → 4)-linked D-galactopyranosiduronic acid esters, where ring-rigidity dictates a *trans* (*ax.*, *ax.*) elimination, and a much slower and more base-dependent β-elimination in polymers consisting of (1 → 4)-linked D-mannopyranosiduronic acid esters, where ring-rigidity dictates a *cis* (*ax.*, *eq.*) elimination.

Bio-Gel P polyacrylamide gel, which had been treated with hydrazine hydrate and then washed with sodium acetate and 4-dimethylaminobenzaldehyde, on stirring with a mixture of alginic acids yielded a matrix suitable for the purification of alginases by affinity chromatography.[101]

Titanium chelates of alginic acid have been prepared, and it was proposed that at least one of the water ligands of the hexaco-ordinated titanium ion had been replaced by a hydroxy-group of the polysaccharide.[102] The chelated alginic acid was used for the immobilization of glucose oxidase, with retention of activity; this process is considered to involve further replacement of a water ligand of the bound titanium ion by either an amino- or a carboxy-group of the protein chain of the enzyme.

Amylopectin.—Amylopectin Azure, a dye derivative of amylopectin available commercially, has been treated with β-amylase to give the β-limit dextrins.[103] The dextrins were shown to be specific substrates for α-amylase, thereby enabling α- and β-amylases to be differentiated.

[99] J. F. Kennedy and H. Cho Tun, *Carbohydrate Res.*, 1973, **26**, 401.
[100] J. N. BeMiller and G. V. Kumari, *Carbohydrate Res.*, 1972, **25**, 419.
[101] V. V. Favorov, *Internat. J. Biochem.*, 1973, **4**, 107.
[102] J. F. Kennedy and C. E. Doyle, *Carbohydrate Res.*, 1973, **28**, 89.
[103] D. E. Bilderback, *Plant Physiol.*, 1973, **51**, 594.

The i.r. spectra of a number of variously nitrated polysaccharides, including amylopectin, have been compared.[27] The range 900—1350 cm^{-1} was found to be useful for distinguishing amylopectin nitrate from the parent polysaccharide, and the use of i.r. spectroscopy for the quantitative determination of nitrate groups in amylopectin nitrate was discussed.

The precipitation of β-limit dextrins of amylopectin and structurally related polysaccharides by salts of quaternary bases has been studied.[104]

Amylose.—Amylose has been labelled radioactively by enzyme-catalysed increase of the chain-length using D-[^{14}C]glucose 1-phosphate as the second substrate.[105] The number of labelled D-glucose residues attached to the amylose molecules was revealed by micromethylation, followed by acid hydrolysis and determination of the radioactivities of the 2,3,4,6-tetra-*O*-methyl- and 2,3,6-tri-*O*-methyl-D-glucoses liberated. The labelled polysaccharide was used as a substrate in a study of the kinetics of β-amylase.

The o.r.d. and c.d. spectra of amylose acetate in the solid state have been recorded.[106] The c.d. spectra of amylose-acetate films, after annealing at various temperatures, were compared with those obtained for the same material in trifluoroethanol solution at various temperatures. Whereas the solution spectra were interpretable in terms of a partial retention of the helical conformation, with increasing destruction of ordered conformations at higher temperatures, the solid-state spectra showed the reverse trend. The observed temperature dependences of the c.d. spectra of both films and solutions were consistent with one another, and *X*-ray diffraction analysis of the films also showed the presence of increasing degrees of crystallinity at the higher annealing temperatures. O.r.d. spectroscopic data were in accord with these results. It was concluded that increasing order in amylose acetate, both in the solid state and in solution, is accompanied by significant changes in the o.r.d. and c.d. spectra, although the exact causes of these changes are not known.

The preparation of 6-chloro-6-deoxyamyloses of various degrees of substitution, using methanesulphonyl chloride in DMF, has been reported.[107] The 6-chloro-6-deoxy derivative could be converted (using sodium azide) into 6-azido-6-deoxyamylose, photolysis of which gave 6-aldehydoamylose. The chlorination procedure is claimed to offer advantages of improved selectivity, procedural convenience, and lower cost compared with a sulphonylation procedure previously described (Vol. 5, p. 395) for the production of 6-aldehydoamyloses.

The structure of the linkage formed when compounds containing amino-groups react with cyclic imidocarbonate derivatives of amylo-oligosaccharides (amylodextrins, malto-oligosaccharides, *etc.*) has been investigated.[26] The mixed oligosaccharides (largely of DP 4—6) were first

[104] B. N. Stepanenko and E. V. Avakian, *Doklady Akad Nauk S.S.S.R.*, 1973, **210**, 975.
[105] E. László, J. Holló, and B. Bánky, *Carbohydrate Res.*, 1972, **25**, 355.
[106] A. Sarko and C. Fischer, *Biopolymers*, 1973, **12**, 2189.
[107] D. Horton, A. E. Luetzow, and O. Theander, *Carbohydrate Res.*, 1973, **27**, 268.

allowed to react with cyanogen bromide to give the cyclic imidocarbonate derivatives, whereafter any change in the net charge on subtilisin on reaction with these derivatives was assessed with the aid of isoelectric focusing. It was considered that conversion of the lysyl residues of the subtilisin protein-chain into *N*-alkylcarbamates is likely to result in loss of positive charges on the protein surface in the pH range 0—10. Since the pK_a of a model N-substituted imidocarbonate, *viz.* ethyl isobutyl imidocarbonate, was found to be 4.7, it was assumed that imidocarbonates are much weaker bases than the protein amino-groups; thus, formation of an N-substituted imidocarbonate would cause a decrease in the number of protonated groups in the pH range 6—10. However, formation of an N-substituted isourea is considered not to influence the charge on the protein, since the dissociation constants of isoureas and protein amino-groups are of the same order of magnitude. Isoelectric focusing of the subtilisin derivatives indicated that isoureas are major products of the reaction of lysyl groups of proteins with polysaccharide cyclic imidocarbonates.

6-Deoxy-6-iodoamylose has been prepared from 2,3-di-*O*-acetylamylose by tosylation at C-6 and treatment of the sulphonate with sodium iodide in DMF.[108] Deacetylation with sodium methoxide in anhydrous methanol then yielded 6-deoxy-6-iodoamylose. A series of variously substituted iodo-derivatives was so prepared for use in a study of the action of amylases on modified substrates. Unfortunately, samples having the highest degrees of substitution were insoluble and, therefore, unsuitable for enzymic studies. A study of the action of α-amylase on 6-deoxy-6-iodoamylose (d.s. 0.037) showed that the enzyme did not liberate 6-deoxy-6-iodo-D-glucose. Chromatography, enzymic analysis, and g.l.c. and mass spectrometry of volatile derivatives of the products indicated that the saccharide of smallest molecular weight liberated by α-amylase is 6^2-deoxy-6^2-iodomaltotriose. It appears that the iodo-group in this trisaccharide has the same effect as the branch point in amylopectin, which yields 6^2-α-maltosylmaltotriose as the smallest fragment of enzymolysis.

The i.r. spectra of amylose nitrated in a variety of ways have been studied.[27]

A study of the grafting of styrene on to amylose has been made using hydrogen peroxide as initiator and cobalt(II), chromium(III), copper(II), iron(II), zinc(II), and silver(I) ions as catalysts.[109] It was found that a maximum grafting yield was obtained when iron(II) ions were employed; the effects of varying the concentrations of the iron(II) ion, hydrogen peroxide, amylose, and monomer, and the temperature and pH were investigated for this system. The optimal conditions for the preparation of graft copolymers of amylose and polystyrene were deduced. For the iron(II)–hydrogen peroxide system, evaluation of the chain-transfer

[108] C. E. Weill and J. Guerrera, *Carbohydrate Res.*, 1973, **27**, 451.
[109] C. M. Patel and V. M. Patel, *Stärke*, 1973, **25**, 12.

constant and analysis of the kinetic data indicated a decrease in the extent of termination of homopolymer and graft-copolymer radicals.[110] The results also showed an increase in the yield of graft copolymer. Acetylation of the graft copolymer rendered it soluble in chloroform.[111] Examination of the acetylated derivative by u.v., i.r., and n.m.r. spectroscopy, solubility techniques, and d.t.a. led to the conclusion that it is a true graft copolymer.

The effect of complexing with iodine on the conformation of amylose in aqueous solution has been studied.[112] Using amylose of molecular weight 8×10^5, viscosity measurements were made with a series of concentrations of iodine. By a novel extrapolation method, the intrinsic viscosities of the amylose–iodine complexes were determined under various conditions of iodine binding. Contrary to a view long held in this field, it was found that the intrinsic viscosities of amylose solutions decrease significantly upon complexing, and it was proposed that this is due to a shortening of the linear dimension of the polymer chain. The change of conformation is apparently caused by contraction of the loose helical regions of the amylose macromolecule owing to entrapment of iodine inside the helical cavities. On the basis of these findings and previous kinetic studies, a new model was suggested for amylose.

The precipitation of β-dextrins of amylopectin and structurally related polysaccharides on complexing with salts of quaternary bases has been studied.[104]

Carrageenans.—Conformational data on λ- and κ-carrageenans in solution have been obtained from c.d. spectroscopic studies and from the induced Cotton effects of their complexes with Methylene Blue.[113] The results showed that the dye molecules bind to κ-carrageenan giving a right-handed exciton-like band that correlates well with the proposed right-handed double-helix for the polysaccharide. The induced Cotton effect for the λ-carrageenan–dye complex is negative, demonstrating a qualitative difference between the preferred conformations of the two polysaccharides. It was noted that comparison of these results with those obtained for glycosaminoglycans could be used to reveal general patterns of conformational preferences and the effects of functional groups on the conformations.

Cellulose.—An article on the present, future, and industrial uses of carbohydrates has discussed derivatives of cellulose.[114] A book recently published on the properties and applications of ion-exchange resins has dealt with their uses in industry, analytical chemistry, pharmacy, and medicine, as well as with the theory of ion exchange; sections on cellulose derivatives suitable for use as anion- and cation-exchangers are included.[115]

[110] C. M. Patel, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1973, **25**, 233.
[111] C. M. Patel and V. M. Patel, *Stärke*, 1973, **25**, 47.
[112] M. B. Senior and E. Hamori, *Biopolymers*, 1973, **12**, 65.
[113] A. L. Stone, *Biopolymers*, 1972, **11**, 2625.
[114] M. Stacey, *Chem. and Ind.*, 1973, 222.
[115] K. Dorfner, 'Ion Exchangers – Properties and Applications', Ann Arbor Science, Ann Arbor, 1972.

The thermal degradation (under nitrogen) of four different forms of cellulose has been studied by using a thermobalance, and weight losses and other physicochemical data were fitted to mathematical equations.[116] It was postulated that thermal degradation occurs by random nucleation and growth in the cellulose fibrils in such a way as to yield a material whose microporous structure replicates that of the pore system of the parent molecule. Thermoanalytical techniques have also been used to determine the kinetic parameters involved in the thermal and thermo-oxidative degradations of cotton and wood celluloses, acetylated, phosphated, and sulphated celluloses, and graft copolymers of cellulose and acrylonitrile having different nitrogen contents.[117] The volatile products were determined quantitatively, and changes occurring in the charring residue were followed by i.r. and e.s.r. spectroscopy. The action of several organic salts on the thermal degradation of cellulose was also investigated, and the effects of various treatments on glycosan formation, thermo-oxidation, and dehydration were discussed.

Exposure of cotton cellulose to hydrochloric acid, chlorine, and u.v. irradiation in carbon tetrachloride led to the formation of metastable, free macroradicals arising from homolytic cleavage of the C-1—H bond.[118] The radicals recombined in the presence of water. The initial stages of the photolysis of cellulose *in vacuo* have been followed by analysis of the gaseous products by mass spectrometry.[119] Only hydrogen, carbon monoxide, and carbon dioxide were formed.

The electro-optical properties of solutions of several cellulose esters in 1,4-dioxan have been investigated.[120] It was shown that the macromolecular esters display high equilibrium and kinetic-chain rigidities. The coincidence of the signs of the Kerr and Maxwell effects for solutions of the samples revealed general relationships exhibited by all rigid-chain polymers for which the longitudinal geometric axis of the molecule (*i.e.* the direction of the greatest geometric length) is also the symmetry axis of its polar and optical properties.

It has been shown that heterogeneous acetylation of cellulose may be self-activated, giving rise to increased reactivity and, accordingly, to increased reaction rate.[121] Both the homogeneity of the reaction and the activation factor determined the quality of the cellulose acetate formed. Characterization of the insoluble remnants, according to the degree of heterogeneous acetylation, showed that D-xylose and D-mannose residues, which are constituents of wood cellulose, are located in those parts of the cellulosic material that are difficult to acetylate.

[116] D. Dollimore and B. Holt, *J. Polymer Sci., Polymer Phys.*, 1973, **11**, 1703.
[117] M. Košik, V. Lužáková, and V. Reiser, *Cellulose Chem. Technol.*, 1972, **6**, 589.
[118] V. I. Ivanov, N. J. Kuznetsova, V. I. Kogan, Z. A. Maslinkovskaia, and A. I. Bosova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **196**, 1352.
[119] A. Bos and A. S. Buchanan, *J. Polymer Sci., Polymer Chem.*, 1973, **11**, 833.
[120] V. N. Tsvetkov, E. I. Riumtsev, I. N. Shtennikova, T. V. Peker, and N. V. Tsetkova, *Doklady Akad. Nauk S.S.S.R.*, 1972, **207**, 1173.
[121] G. A. Petropavlovskiy and A. F. Zaitseva, *Cellulose Chem. Technol.*, 1972, **6**, 319.

Although calcium ions have been shown to bind to matrices of poly-(D-glucose) with low affinity, they do so in amounts sufficient to influence the rate of dialysis of calcium ions across cellulose membranes.[122] In applications where this interaction is critical, it has been found that the binding of calcium ions can be largely prevented by acetylation of the membrane.

Cellulose gel has been acetylated with acetic anhydride to d.s. 1.55, methylated with dimethyl sulphate to d.s. 1.9, and trimethylsilylated with trimethylsilyl chloride to d.s. 2.7 with retention of its gel-swelling properties.[123] The partitioning behaviour in DMF of homologous series of polar and non-polar compounds of low molecular weight on the product and cellulose gels has been studied. The nature of the functional groups present in the gel matrix, solute, and solvent markedly affected the partitioning of the solute, which was discussed in terms of the structure of the components.

An attempt has been made to evaluate the distribution of acetyl groups in cellulose acetates of different molecular weights by means of t.l.c.[124] The fine structure of acetylated cellulose fibres has been investigated by measurements of the density and swelling properties.[125]

The crystalline structures of cellulose acetates and saponified products from various crystalline forms of modified cellulose have been investigated.[126] A new transition of cellulose acetate in deuteriochloroform solution has been revealed by ^{1}H n.m.r. spectroscopy.[127] The molecular mechanism of this transition appears to be associated with a chair–boat conformation change in the pyranose ring. The structures of desalination membranes composed of cellulose acetate have been investigated.[128] The aim of the work was to determine whether orientation of the polymer in the plane of the membrane or perpendicular to the surface of the membrane affects the flux and salt-rejection properties.

Several types of cellulose acetate were found to be far more readily hydrolysed than natural cellulose or cellulose regenerated from cellulose acetates.[129] It was suggested that the ease of hydrolysis of cellulose acetates might be due principally to an alteration in the crystallite (micelle) structure resulting from the introduction of acetyl groups.

The nature and behaviour of free radicals induced in acetylated cotton celluloses on γ-irradiation have been studied by e.s.r. spectroscopy.[130]

[122] K. C. Reen, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 1136.
[123] K. Chitumbo and W. Brown, *J. Chromatog.*, 1973, **80**, 187.
[124] K. Kamide, S. Manabe, and E. Osafune, *Makromol. Chem.*, 1973, **168**, 173.
[125] Y. Fahmy and F. Mobarak, *Svensk. Papperstidn.*, 1972, **75**, 853.
[126] J. Hayashi, A. Sueoka, and S. Watanabe, *Nippon Kagaku Kaishi*, 1973, 160.
[127] K. Ogura, H. Sobue, M. Kasuga, and S. Nakamura, *J. Polymer Sci., Polymer Letters*, 1973, **11**, 421.
[128] R. D. Sudduth and C. E. Rogers, *J. Polymer Sci., Polymer Letters*, 1973, **11**, 603.
[129] T. Nakai and H. Demura, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1537.
[130] P. K. Chidambareswaran, V. Sundaram, and B. B. Singh, *J. Polymer Sci., Part A-1 Polymer Chem.*, 1972, **10**, 2655.

Dehydrogenation and deacetylation appeared to be responsible for the production of free radicals from samples irradiated at 77 K. The degree of substitution of cellulose acetate enhanced the yield of acetyl radicals on irradiation at 77 K, but adversely affected the overall concentration of radicals when irradiation was carried out at 300 K. The e.s.r. spectra of the samples irradiated at 300 K *in vacuo* were more intense than those irradiated in air. The nature, yield, and post-irradiation behaviour of the primary radicals were discussed in the light of chemical effects ultimately observed.

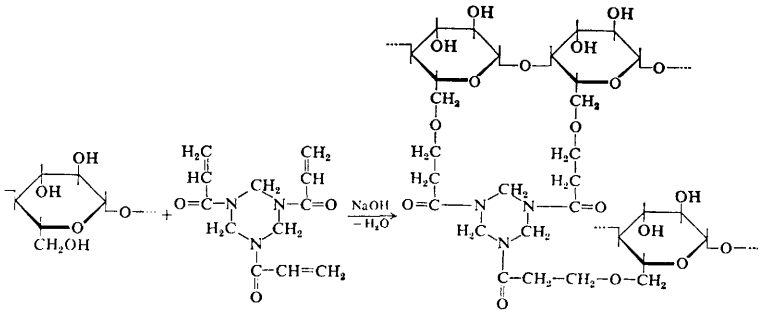
The process of grafting acrylonitrile and methacrylic acid onto cellulose acetate using dicyclohexylperoxydicarbonate as initiator has been studied.[131] In the case of acrylonitrile, the extent of grafting increased with increasing concentration of initiator, whereas the opposite was true in the case of methacrylic acid. The formation of the graft copolymer of methacrylic acid and cellulose acetate involved the generation of methacrylic acid free-radicals, homopolymerization of methacrylic acid, and grafting by a chain-transfer mechanism.

The adsorption of cellulose acetate from chloroform solution on to calcium silicate was found to follow first-order kinetics.[132] The adsorption data were adequately described by the Langmuir isotherm, indicating that the polymer behaves as a rigid molecule in the adsorbed state. An attempt was made to fractionate a sample of cellulose acetate by this adsorption technique.

Cellulose acetate and ethylcellulose have been used as matrices for the production of fibre-entrapped enzymes by a wet-spinning process.[133] In this process, the pore size of the cellulosic fibres could be controlled, and the enzyme remained entrapped within the pores. Active, immobilized derivatives of β-fructofuranosidase and several other enzymes, and mixed derivatives of β-fructofuranosidase, glucose oxidase, and catalase, and of glucose oxidase and peroxidase were produced, and it was demonstrated that the fibres could be woven into a cloth. Mixing a solution of cellulose acetate in methylene chloride with an aqueous solution of glucoamylase to form an emulsion has been used to prepare an immobilized form of the enzyme, which was also obtained by wet-spinning of the emulsion.[134] Parameters affecting the activity of the glucoamylase fibres (including the enzyme content of the fibres) and a possible use for the immobilized enzyme in converting starch into D-glucose were investigated.

Michael addition of cellulose to the αβ-unsaturated ketone (37) has been investigated and conditions for the reaction leading to the product (38) have been defined.[135]

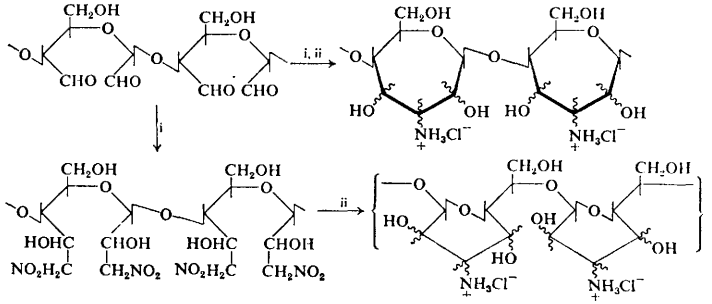
[131] V. A. Landysheva, I. D. Shamolina, T. V. Zhigalova, G. I. Antipova, and V. E. Lozkhin, *Cellulose Chem. Technol.*, 1972, **6**, 203.
[132] H. C. Trivedi, V. M. Patel, and R. D. Patel, *European Polymer J.*, 1973, **9**, 525.
[133] D. Dinelli, *Process Biochem.*, 1972, **7**, No. 8, p. 9.
[134] C. Corno, G. Galli, F. Morisi, M. Bettone, and A. Stopponi, *Stärke*, 1972, **24**, 420.
[135] D. G. Dimitrov, T. S. Khristov, S. A. Todorova, and S. K. Karaivanova, *Cellulose Chem. Technol.*, 1972, **6**, 307.



(37) (38)

Periodate-oxidized cellulose has been condensed with nitromethane, whereafter reduction of the product gave amino-polyacetals (Scheme 15).[136]

A general method for the preparation of 6-aldehydo- and 6-carboxy-celluloses has been reported (see Scheme 16).[137] 6-Chloro-6-deoxycellulose (39), prepared by treating cellulose with methanesulphonyl chloride in DMF, was converted into the corresponding 6-azido-6-deoxy-derivative



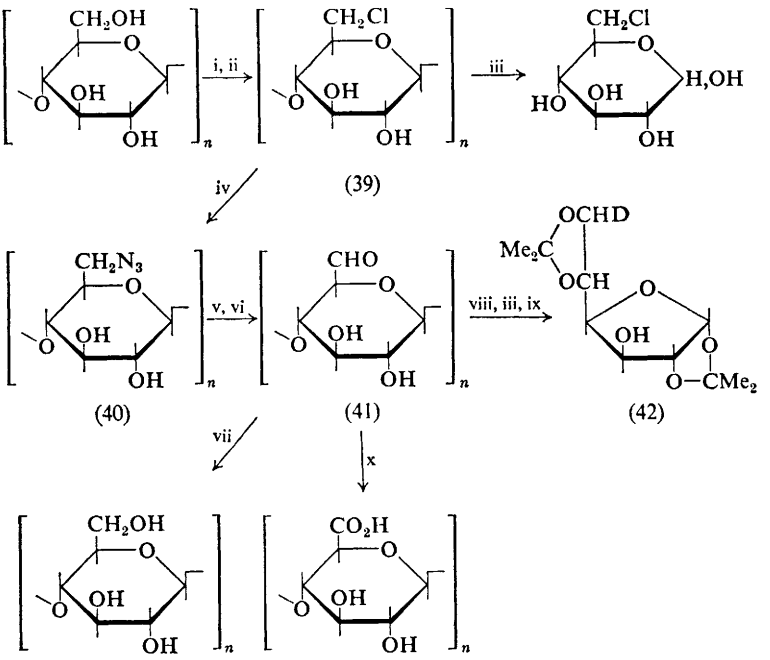
Reagents: i, $MeNO_2$; ii, Ni–H_2

Scheme 15

(40), which on photolysis and mild hydrolysis yielded the aldehydic derivative (41). The degree of substitution of the aldehydic derivative was deduced from a determination of the chlorine content of 6-chloro-6-deoxycellulose, which could be hydrolysed by acid to 6-chloro-6-deoxy-D-glucose. 6-Aldehydocellulose (41) was characterized by determination of its copper number, by reduction to cellulose, by oxidation to 6-carboxycellulose, and by deuteriation (with $NaBD_4$), hydrolysis, and acetonation to give the diacetal (42), which was identified by mass spectrometry. The deuterium content of diacetal (42) also afforded a measure of the degree of substitution of 6-aldehydocellulose.

[136] Z. I. Kuznetsova, E. G. Ivanova, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 1858.

[137] D. Horton, A. E. Luetzow, and O. Theander, *Carbohydrate Res.*, 1973, **26**, 1.



Reagents: i, MsCl–DMF; ii, Na_2CO_3–H_2O; iii, H_3O^+; iv, N_3^-; v, *hv*; vi, pH 4; vii, $NaBH_4$; viii, $NaBD_4$; ix, Me_2CO–$CuSO_4$; x, $NaClO_2$

Scheme 16

6-Amino-6-deoxycellulose (d.s. 0.89) has been prepared from 6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoylcellulose by reduction with lithium aluminium hydride.[138] A diaminodideoxy-derivative of cellulose (d.s. 1.87) was similarly prepared from a cellulose ditosylate *via* azide exchange, *etc.*

4-Aminobenzylcellulose (in diazotized form) and carboxymethylcellulose azide have been used for the immobilization of aspartate ammonia-lyase by covalent attachment to the matrix.[139] DEAE- and triethylaminoethyl-celluloses have also been used for the immobilization of aspartate ammonia-lyase, but the enzyme–matrix bonds in these cases are ionic in nature. Each of the cellulose derivatives was investigated for use in the continuous production of L-aspartic acid. Lysozyme has also been immobilized by reaction with diazotized 4-aminobenzylcellulose.[140] Despite the fact that a covalent bond is formed, attachment of the enzyme was found to be partly reversible.

[138] A. I. Usov, N. I. Nosova, S. I. Firgang, and O. P. Golova, *Vysokomol. Soedinenii*, 1973, **A15**, 1150.
[139] T. Tosa, T. Sato, T. Mori, Y. Matuo, and I. Chibata, *Biotechnol. Bioeng.*, 1973, **15**, 69.
[140] R. Datta, W. Armiger, and D. F. Ollis, *Biotechnol. Bioeng.*, 1973, **15**, 993.

Electro-optical properties of solutions of benzylcellulose, ethylcellulose, and various cellulose esters [butyrate, benzoate, phenylcarbamate, diphenylacetate, and diphenylphosphone carbamate (43)] in 1,4-dioxan have been



(43)

investigated.[141] It was found that the macromolecules display high equilibrium and kinetic-chain rigidities. The coincidence of the Kerr and Maxwell effects for the samples in solution revealed general relationships exhibited by all rigid-chain polymers for which the longitudinal geometric axis of the molecule (*i.e.* the direction of the greatest geometric length) is also the symmetry axis of its polar and optical properties.

Cellulose has been converted into a bromoacetylated form by reaction with bromoacetyl bromide, and the bromo-ester was transformed into iodoacetylcellulose on treatment with sodium iodide.[142] Coupling of glucoamylase to the halogenoacetylcelluloses, without pretreatment with organic solvents, led to the formation of large particles of immobilized glucoamylase possessing low, but specific, activity. However, pre-treatment of the halogenoacetylcelluloses with organic solvents yielded fine particles possessing high activity and retaining most of the activity of the enzyme against maltose. Investigation of the size of the halogenated celluloses in the coupled products showed that maximal enzymic activity was evinced by the smallest particles.

The preparation of water-insoluble carbonate derivatives of cellulose by reaction with ethyl chloroformate has been further investigated.[99] Cellulose was used in various forms (microcrystalline powder, sheet, and shredded paper) and the reaction was extended to the analogous derivatization of DEAE-cellulose and other polysaccharides. The presence of *trans*-2,3-cyclic carbonate and acyclic carbonate (ethyloxycarbonyl) groups in the products was revealed by i.r. spectroscopy. It appears that the relative proportions of cyclic and acylic groups in the derivatives is a function of the reaction conditions rather than of the availability of primary hydroxy-groups at C-6. The carboxy-groups in carboxymethylcellulose prevented the formation of a *trans*-2,3-cyclic carbonate, but this effect could be overcome by esterification.

The reaction of cellulose *trans*-2,3-cyclic carbonate with amino- and mercapto-compounds has been studied.[143] Non-sulphurous amino-acids, albumin, sodium 4-aminosalicylate, *N*-acetylcysteine, and 2-aminoethanethiol are considered to react by nucleophilic attack of the amino- or

[141] V. N. Tsvetkov, E. I. Rjumtsev, I. N. Shtennikova, T. V. Peker, and N. V. Tsvetkova, *European Polymer J.*, 1973, **9**, 1.
[142] H. Maeda and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1581.
[143] J. F. Kennedy and H. Cho Tun, *Carbohydrate Res.*, 1973, **29**, 246.

sulphydryl-groups on the cyclic carbonate. Cellulose *trans*-2,3-cyclic carbonate, which had been allowed to react with 4-aminosalicylate, was able to complex with streptomycin; however, much larger amounts of the antibiotic were taken up by the borate complex of the salicylyl derivative, whereas native cellulose bound only small amounts of streptomycin, even after pre-treatment with borate.

Cellulose *trans*-2,3-cyclic carbonate has continued to be used for the immobilization of enzymes and the applications of this cellulose derivative in modified paper form have been discussed.[99] In a study of the coupling of β-glucosidase and trypsin to cellulose *trans*-2,3-cyclic carbonate, it was found that retention of activity by the enzyme on coupling could be improved by a high degree of substitution and by adjustment of the concentration of protein and the ratio of the reactants.[144] Bound trypsin exhibited a higher activity at low pH values than did the free enzyme.

The activities of immobilized dextranases, produced by attaching the enzyme covalently to cellulose cyclic carbonate, carboxymethylcellulose azide, or cellulose *trans*-2,3-cyclic imidocarbonate, have been compared.[145] Increased stability of the enzyme was noted in each case.

The application of cellulose *trans*-2,3-cyclic carbonate to the preparation of water-insoluble immunoadsorbents for use in the purification of antibodies to immunoglobulins has been described.[40] Using immunoglobulin IgG and IgM and their respective antisera, it was found that coupling of the antigen occurred under mild conditions to produce insoluble reagents that were stable, highly specific for the antibody, and possessing very high antibody-binding capacities with respect to the amount of antigen coupled. Up to 86% of the adsorbed antibody could be recovered in purified form, and this allowed repeated use of the cellulose derivatives for the large-scale purification of antibodies. The cellulose-IgG derivative was demonstrated to be more efficient than equivalent preparations of the immunoglobulin coupled to macroporous agarose cyclic imidocarbonate. The use of cellulose *trans*-2,3-cyclic carbonate as a matrix for the insolubilization of antigens and antibodies for the purpose of radioimmunoassay has been reported.[146] Antibodies to follicle-stimulating hormone retained their immunological reactivity on reaction with the cellulose derivative, and the product was suitable for the solid-phase radioimmunoassay of unknown amounts of the hormone by competitive binding; low, non-specific adsorption characteristics gave this system advantages over other systems. Cellulose *trans*-2,3-cyclic carbonate was also used to immobilize follicle-stimulating hormone with retention of the immunological activity.

The coupling of a number of antibiotics to cellulose and cellulose *trans*-2,3-cyclic carbonate under a variety of conditions has been investigated, and it was shown that active, insoluble derivatives of the antibiotics

[144] J. F. Kennedy and A. Zamir, *Carbohydrate Res.*, 1973, **29**, 497.
[145] N. W. H. Cheetham and G. N. Richards, *Carbohydrate Res.*, 1973, **30**, 99.
[146] J. F. Kennedy and H. Cho Tun, *Carbohydrate Res.*, 1973, **30**, 11.

could be produced.[147] Although the antibiotics bound firmly to cellulose, the use of cellulose carbonate extended the range of antibacterial activity retained. It is considered that physical adsorption phenomena are operating with cellulose, whereas a covalent bond is more likely to be formed in the case of the carbonate derivative. Release of the antibiotics from the matrix during testing for antibacterial activity is believed to occur through the action of a cellulase and, additionally, an esterase in the case of cellulose *trans*-2,3-cyclic carbonate. These new types of derivative appear to offer new outlets for the commercial use of cellulose.

The degree of substitution of macroporous cellulose obtained with ethyl chloroformate has been controlled by moderating the reaction by addition of small quantities of water.[148] This procedure was used in the preparation of a matrix whose physical and chemical properties allowed the covalent binding of chymotrypsin in such a way that the activity of the insolubilized enzyme was appreciable towards substrates of high and low molecular weight.

Experimental conditions for a study of the electro-optical properties of carboxymethylcellulose (as a model polyelectrolyte) have been determined for a specific carboxymethylcellulose (d.s. 1.3, DP 180).[149] The transient Kerr effect was found to be a function of the concentration of the polysaccharide derivative and of the field and ionic strengths. The optical anisotropy was shown to be independent of ionic strength, but the electrical anisotropy decreased sharply with increasing ionic strength. The results obtained were discussed in relation to polarization theories of polyelectrolytes. The molecular dimensions of carboxymethylcellulose, calculated from birefringence kinetics, suggested that the molecule is a rigid rod. A study of the dependence on concentration of the reduced viscosity of carboxymethylcelluloses in the absence of added salts has led to the definition of two parameters typical of the polyanion.[150] The molecular dimensions of the free acid and sodium forms of carboxymethylcellulose were determined from these parameters using a rod-like model. The gradient dependence of viscosity was also studied, and two samples (molecular weights 4.1×10^4 and 1.8×10^5) in aqueous solution showed the non-Newtonian behaviour typical of rigid particles.

Carboxymethylcellulose has been shown to interact with a cellulase, and can be used as a matrix for affinity chromatography of the enzyme.[151] That this interaction is solely of an affinity type was indicated by the fact that the enzyme, which also appears to be unusually acidic, is not bound by carboxymethyl-Sephadex. Carboxymethylcellulose exhibited a high affinity for an α_1-glycoprotein from human serum, and cellulose phosphate loaded

[147] J. F. Kennedy and H. Cho Tun, *Antimicrobial Agents and Chemotherapy*, 1973, **3**, 575.
[148] J. F. Kennedy, S. A. Barker, and A. Rosevear, *J.C.S. Perkin I*, 1973, 2293.
[149] M. Hanss, B. Roux, J. C. Bernengo, M. Milas, and M. Rinaudo, *Biopolymers*, 1973, **12**, 1747.
[150] M. Moan and C. Wolff, *European Polymer J.*, 1973, **9**, 1085.
[151] J. J. Marshall, *J. Chromatog.*, 1973, **76**, 257.

with nickel(II) ions has been used in the affinity chromatography of this glycoprotein.[32]

Carboxymethylcellulose, which had been converted into the azide, and cellulose *trans*-2,3-cyclic imidocarbonate have been used for the production of an immobilized derivative of pronase.[152] The azide derivative has also been used for the immobilization of chymotrypsin[153] and aspartate ammonia-lyase.[139]

The free carboxy-groups in carboxymethylcellulose are able to prevent the formation of the *trans*-2,3-cyclic carbonate on treatment with ethyl chloroformate.[99]

Cyanoethylcellulose with a low degree of substitution has been prepared from various types of cellulosic material by a two-step procedure using acrylonitrile in aqueous alkali.[154] However, the degree of substitution obtained by this procedure is reported to be much lower than that obtained by reaction of cellulose with an excess of acrylonitrile.[155] Conditions conducive to cyanoethylation were examined, and it was found that, during the course of cyanoethylation, nitrile groups were hydrolysed to carboxy-groups. The accessibility of the cellulose and the reaction temperature had a considerable influence on the degree of substitution achieved, particularly when an excess of acrylonitrile was used.[156]

DEAE-cellulose (prepared by treatment of cellulose with a mixture of monochloroacetic acid and chloroacetyl chloride, and reaction of the resulting chloroacetylcellulose with diethylamine) has been used to prepare an immobilized derivative of β-fructofuranosidase.[157] The bond between the polysaccharide derivative and the enzyme is ionic, but it was shown to be stable to electrolytes in the pH range 5—7. The binding of a cellulase to DEAE-cellulose is considered to be of the affinity type, rather than of the ionic type, and the polysaccharide was used in the purification of the enzyme by affinity chromatography.[158] DEAE-, triethylaminoethyl-, and ECTEOLA-celluloses have been used for the immobilization of aspartate ammonia-lyase.[139]

DEAE-cellulose reacted with ethyl chloroformate to give the *trans*-2,3-cyclic carbonate derivative,[99] which was used for the production of an immobilized form of β-glucosidase.[159] The optimum pH (9—10) for coupling of the enzyme is higher than that (7—8) reported for cellulose *trans*-2,3-cyclic carbonate (see ref. 144, and Vol. 5, ref. 1128). The enzyme is considered to be highly charged, since the pH of coupling is well above its isoelectric point (pI 5.8), and it appears that the enzyme is first adsorbed

[152] A. B. Patel, R. O. Stasiw, H. D. Brown, and C. A. Ghiron, *Biotechnol. Bioeng.*, 1972, **14**, 1031.
[153] V. I. Surovtsev, L. V. Kozlov, and V. K. Antonov, *Biokhimiya*, 1972, **37**, 1139.
[154] J. Pastýr and L. Kuniak, *Cellulose Chem. Technol.*, 1972, **6**, 197.
[155] M. Pašteka, A. Piklerová, and A. Pikler, *Cellulose Chem. Technol.*, 1973, **7**, 23.
[156] M. Pašteka, A. Pikler, and A. Piklerová, *Cellulose Chem. Technol.*, 1973, **7**, 31.
[157] H. Maeda, H. Suzuki, and A. Sakimae, *Biotechnol. Bioeng.*, 1973, **15**, 403.
[158] J. J. Marshall, *Comp. Biochem. Physiol.*, 1973, **44B**, 981.
[159] C. J. Gray and T. H. Yeo, *Carbohydrate Res.*, 1973, **27**, 235.

onto the cellulose derivative. This produces a high concentration of enzyme in the vicinity of the cyclic carbonate groups and leads to an enhanced rate of coupling. The pH–activity profile of the immobilized enzyme was shifted from the normal position in accord with the theory that, because of the ionic groups present on the matrix, the effective pH in the vicinity of the coupled enzyme is different from that of the exterior solution.

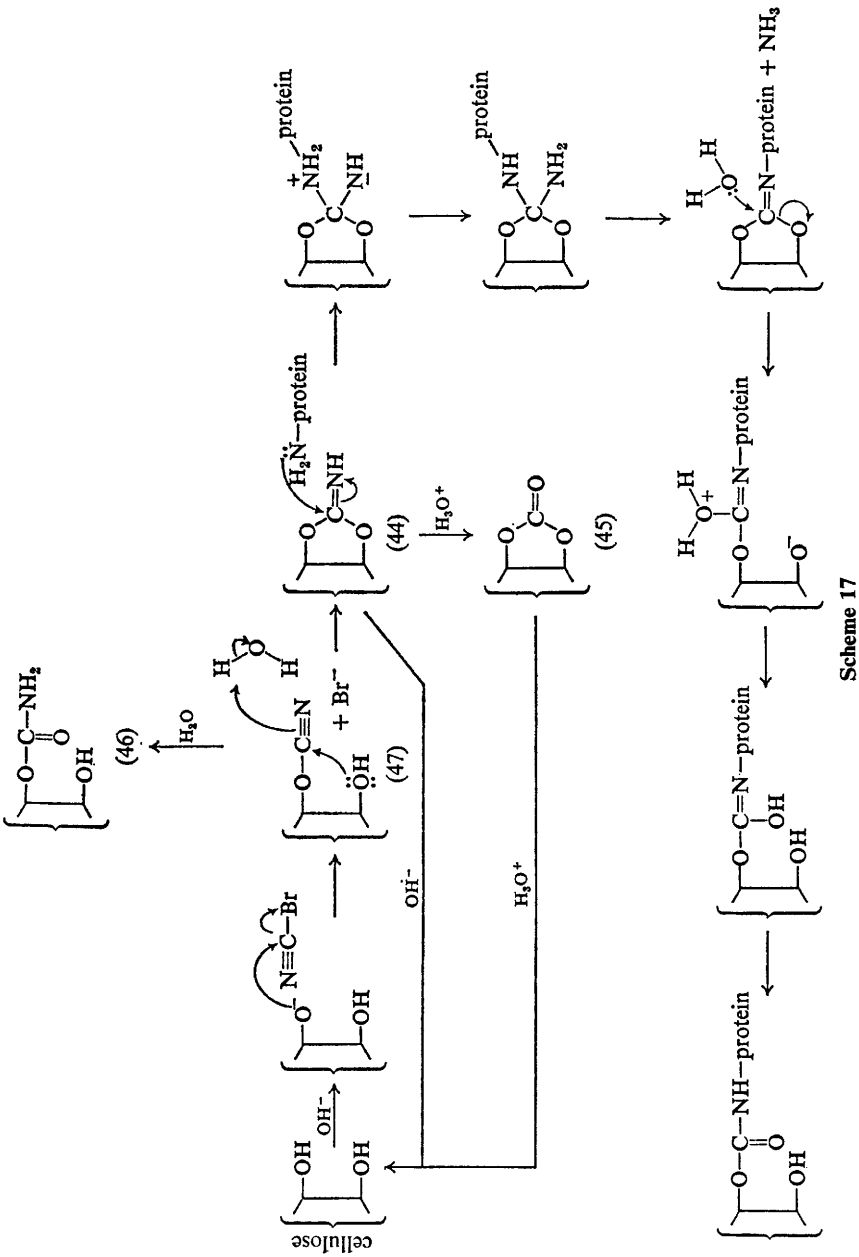
DEAE-cellulose has been derivatized with 2-amino-4,6-dichloro-*s*-triazine, and the product reacted with penicillin amidase to give an immobilized form of the enzyme used for the conversion of benzylpenicillin into 6-aminopenicillanic acid.[160]

DEAE-cellulose, cellophane, and sheets of cellulose and aminated paper have all been used as matrices for the immobilization of enzymes.[96] β-Galactosidase, α-amylase, glucose oxidase, and other enzymes were immobilized in these matrices by cross-linking with glutaraldehyde.

The i.r. spectra of highly substituted ethyl and methyl ethers of cellulose have been determined in deuterium oxide before and after gelatinization.[161] Solubilization of the cellulose ethers in water caused a shift in the C—H band for the alkyl groups, which was attributed to hydrophilic interactions of the alkyl groups in water. It was noted that a highly substituted ethylcellulose (DP 300) could be dissolved in water.

A study of the mechanism of activation of cellulose by cyanogen bromide, to give what is generally supposed to be cellulose *trans*-2,3-cyclic imidocarbonate, and the reaction of the product with proteins (Scheme 17) have been reported (see also pp. 509 and 511).[162] Cellulose treated with cyanogen bromide gave a product exhibiting an i.r. band at 1730 cm^{-1} that is not present in the original material. Brief hydrolysis of the treated material in acid produced an additional, strong band at 1810 cm^{-1}, which disappeared on prolonged acid treatment although the band at 1730 cm^{-1} remained. These and other findings are consistent with the formation of a cyclic imidocarbonate (44), which on treatment with acid gives the acid-labile *trans*-2,3-cyclic carbonate (45). Further studies using cyclohexane-*trans*-1,2-diol and cyclohexyl methanol confirmed these conclusions and indicated that very small amounts of carbamate (46) (0.1%) are formed by way of the cyanate (47). The imidocarbonate derivative was found to bind more protein than cellulose *trans*-2,3-cyclic carbonate obtained from it. Substitution reactions on the carbamate (46) by nucleophilic groups on the protein are considered to occur slowly under the mild conditions employed in the binding of proteins and do not appear to be of significance. It was noted, however, that the exact nature of the products resulting from the coupling of proteins to the cyclic imidocarbonate is still unknown.

[160] D. Warburton, P. Dunnill, and M. D. Lilly, *Biotechnol. Bioeng.*, 1973, **15**, 13.
[161] G. A. Petropavlovskiy, M. M. Kruntchak, and G. G. Vasiljeva, *Cellulose Chem. Technol.*, 1972, **6**, 135.
[162] G. J. Bartling, H. D. Brown, L. J. Forrester, M. T. Koes, A. N. Mather, and R. O. Stasiw, *Biotechnol. Bioeng.*, 1972, **14**, 1039.



Scheme 17

Cellulose *trans*-2,3-cyclic imidocarbonate has been used for immobilization of polyribonucleotide nucleotidyltransferase[36] and glucoamylase.[163] In the latter case, the influence of the surface structure and particle size of the cellulose on the specific activity of the coupled enzyme was investigated; it was found that grinding of the cellulose to give deep cuts in the surface increased the specific activity and the protein content of the product.

In a study of the effects of sucrose and citric acid on the viscosimetric behaviour of aqueous solutions of methylated cellulose, it was found that the flow of a mixture of the three components is thixotropic.[164] This behaviour could be expressed by the law $S = kD^n$, where S is the shearing stress and D is the rate of shear. The differential viscosity of the mixture was decreased on adding citric acid, whereas it was increased on adding sucrose, except at very high concentrations. It was concluded that citric acid tends to rupture the intermolecular hydrogen bonds of methylcellulose molecules, whereas the hydrogen bonds are maintained by sucrose.

The rapid decrease in viscosity of solutions of methylcellulose in the presence of periodate ion has been compared with the behaviours of other polysaccharides and polymers under the same conditions.[25] A mechanism for the cleavage, based on the known disproportionation of ether-type free radicals induced by hydroxy-radicals in periodate solution, has been proposed (see Scheme 13).

The reaction of cellulose with large amounts of 1-naphthyl isocyanate furnished naphthylcarbamoylcellulose (d.s. $\leqslant 3$).[165] This derivative and tritylcellulose (d.s. 0.23) were used in the synthesis of oligonucleotides.

The i.r. spectra of cellulose nitrates, prepared in various ways, have been compared, and the use of i.r. spectroscopy in quantitative determination of the nitrate groups was assessed.[27] The preparation of cellulose nitrates (using mixtures of nitric and sulphuric acids) of different degrees of substitution has also been described.[166] The limiting viscosity number of the cellulose nitrates increased linearly, but slowly, with nitrogen contents between 11.11 and 12.75%, and then at a much steeper rate up to 14.14%N. This effect was explained in terms of the decreasing flexibility of the cellulose nitrate molecule. The viscosity characteristics of cellulose nitrates were examined in a wide range of solvents.

The introduction of nitrate groups into cellulose on treatment with nitrogen dioxide has been studied.[167] Cellulose derivatives, soluble in water and in dilute alkali, have been prepared by heating a bleached sulphite pulp in solutions of nitrogen dioxide in DMF.[168] No oxidation

[163] H. Maeda and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1839.
[164] N. Iso and D. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1467.
[165] P. J. Cashion, M. Fridkin, K. L. Agarwal, E. Jay, and H. G. Khorana, *Biochemistry*, 1973, **12**, 1985.
[166] B. Enoksson, *Chem. Scripta*, 1973, **4**, 43.
[167] I. A. Bashmakov, F. N. Kaputsky, S. V. Baran, and I. N. Ermolenko, *Doklady Akad. Nauk Belorussk. S.S.R.*, 1973, **17**, 734.
[168] L. P. Clermont and F. Bender, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1972, **10**, 1669.

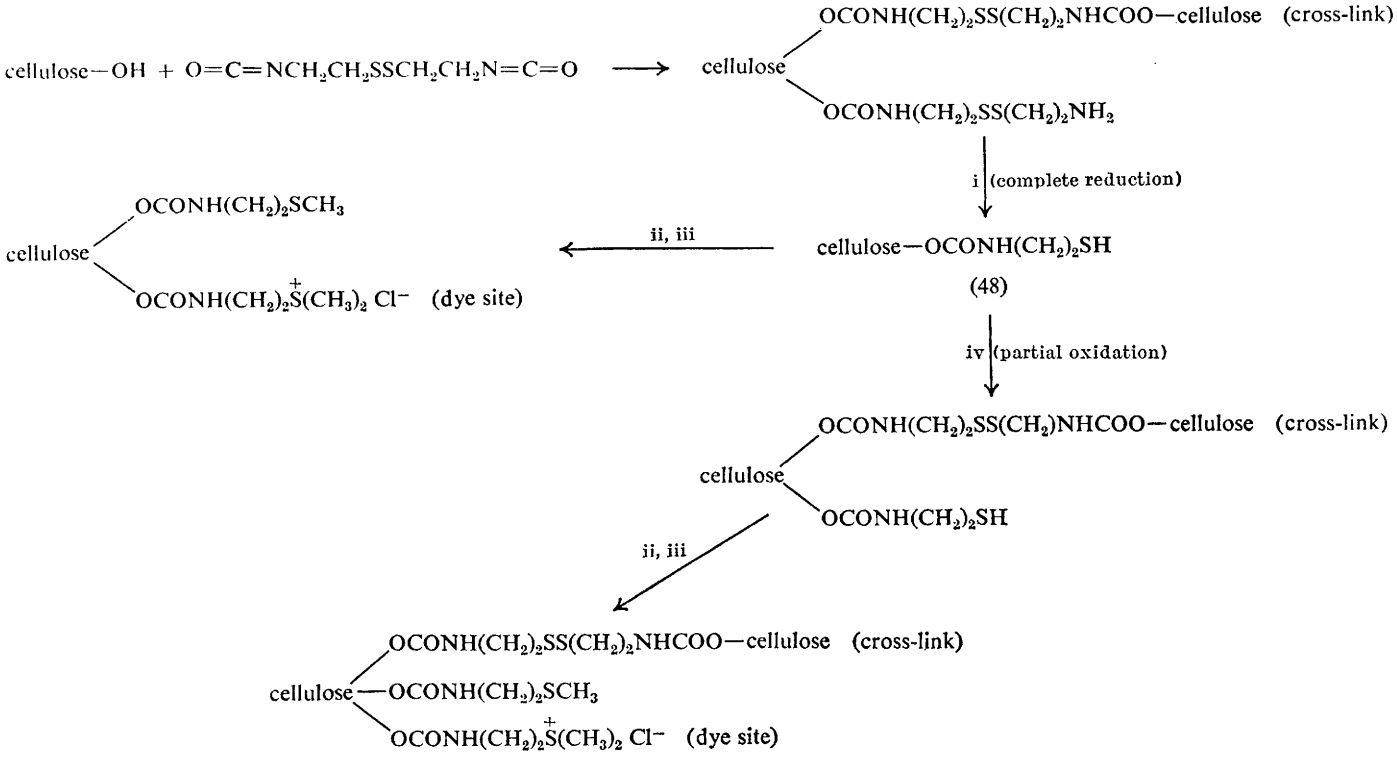
of the polysaccharide was detected, but nitrite and nitrate esters were formed with degrees of substitution ranging from 0.18 to 0.55. The viscosities of aqueous solutions of the nitrated polysaccharide increased with increasing nitrogen content.

A series of degraded cotton celluloses has been nitrated, and osmotic pressure and gel-permeation chromatography measurements have been made in THF.[169] Peak-elution volumes from the permeation experiments were converted into the corresponding extended chain-lengths, using a calibration curve obtained with polystyrene standards, and an effective density factor ($\overline{Q}$) for each sample was obtained by dividing the osmometrically obtained molecular weight by the effective chain-length. The value of $\overline{Q}$ is apparently independent of the molecular size and DP distribution of the nitrated samples. Calculation of the average molecular weight of cellulose nitrate from the permeation data was made using $\overline{Q}$ (instead of the conventional Q-factor), and values for $\overline{M}_w$ and $\overline{M}_v$ were found to be comparable to those obtained by conventional methods. $\overline{M}_n$ could also be obtained by determining the peak-elution volume, converting it into the corresponding extended chain-length, and multiplying the result by $\overline{Q}$. Such calculations were applied to published data and gave results in agreement with the reported values. Comments on this work [170] and a reply [171] have been published.

Ultra-thin membranes of cellulose nitrate have been used for the immobilization of asparaginase within semipermeable microcapsules,[172] and catalase and urease have also been encapsulated in collodion membranes.[173]

In a study of reversible cross-linking in cellulose, unmercerized cotton cellulose was treated with bis(β-isocyanatoethyl)disulphide in DMF (Scheme 18), and the product was reduced to cellulose β-mercaptoethylaminocarboxylate (48).[174] The reduced material was treated with an excess of methyl iodide either directly, or after partial oxidation with iodine in solvents of different swelling power (*e.g.* DMF, methanol, or benzene) to yield partially disulphide-cross-linked samples having approximately the same proportions of cross-linkages and sulphonium groups. The effects of the solvents used on the equilibrium and kinetics of dyeing of these cellulose derivatives with three dyes (Orange II, Direct Scarlet B, and Direct Sky Blue A) of different molecular size were investigated. A 1 : 1 ratio of ionic binding was observed at equilibrium between the sulphonic acid groups of the dye and the sulphonium groups in the modified cottons with the two former dyes. The equilibrium of the other, larger dye was, however, markedly influenced by the solvent used in the cross-linking process; the higher the degree of swelling, the larger was the uptake of dye at equilibrium.

[169] M. Chang, *Tappi*, 1972, **55**, 1253.
[170] L. Segal, J. I. Wadsworth, and J. D. Timpa, *Tappi*, 1973, **56**, 172.
[171] M. Chang, *Tappi*, 1973, **56**, 173.
[172] T. M. S. Chang, *Enzyme*, 1972/3, **14**, 95.
[173] A. O. Mogensen and W. R. Vieth, *Biotechnol. Bioeng.*, 1973, **15**, 467.
[174] M. Sakamoto, Y. Yamada, and H. Tonami, *J. Polymer Sci., Polymer Chem.*, 1973, **11**, 1961.



Reagents: i, Bu^n_3P; ii, MeI; iii, anion exchange; iv, I_2

Scheme 18

The diffusion rate of Direct Scarlet B was also affected by the solvent used: the higher the degree of swelling, the higher was the diffusion rate. The pore structure of the cross-linked cellulose was discussed, and it appears that the polysaccharide derivative possesses a 'memory' of its physical state at the time of cross-linking, to which it has a tendency to revert when placed in a solution of a dye.

Interest has continued in the preparation of nucleic acid derivatives of cellulose, which are obtained simply by heating a nucleic acid (or related compound) with cellulose. Oligo(deoxythymidylic acid)- and oligo(deoxycytidylic acid)-celluloses, prepared in this way, have been used in the separation and synthesis of ribonucleic acids;[175] similarly, (deoxyribonucleic acid)-cellulose has been used in the separation of the RNA polymerase holoenzyme from core enzyme.[176] Several celluloses have been tested for their abilities to bind homonucleotide oligomers and naturally occurring ribonucleic acids.[177] The binding to unesterified cellulose was shown to be much less specific than that for celluloses esterified with oligo(deoxythymidylic acid) and oligo(deoxycytidylic acid). Since the various cellulose preparations differed in their abilities to bind the oligonucleotides and ribonucleic acids, it was suggested that an impurity present in cellulose is responsible for the binding properties. Treatment of the celluloses with sodium bisulphite reduced the amount of poly(adenylic acid) bound, indicating that binding is due to a lignin-like contaminant.

Sulphopropylcellulose has been prepared by alkylation of cellulose with propanesulphone.[178] The distribution of the substituents was determined by identification of the *O*-sulphopropyl-D-glucoses released on hydrolysis. The molecular weights and intrinsic viscosities of sulphopropyl- and sulphobutyl-celluloses in alkaline solution were estimated from sedimentation and viscosimetric measurements. Mark–Howink equation constants were also calculated for the solutions.[179]

Treatment of 6-*O*-tritylcellulose with DMSO gave an oxidized material that, on reduction, gave rise to mannoglucans containing up to 30% of D-mannose.[180]

A new process has been reported for the manufacture of viscose in which alkali cellulose is subjected to re-steeping and re-pressing after ageing, but prior to xanthation.[181] If the concentration of the re-steeping liquor was maintained at 10—12%, it was found that xanthation could be effected with amounts of carbon disulphide considerably less than normally used, without impairing the solubility of the xanthate or the ability to filter the

[175] J. DeLarco and G. Guroff, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 1233.
[176] R. Mukai and Y. Iida, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 134.
[177] J. DeLarco and G. Guroff, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 486.
[178] A. I. Usov, Z. I. Kuznetsova, and V. S. Arkhipova, *Vysokomol. Soedinenii*, 1973, **15B**, 147.
[179] V. S. Arkhipova, Z. I. Kuznetsova, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 2124.
[180] A. I. Usov and V. S. Ivanova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1973, 910.
[181] H. Sihtola and T. Rantanen, *Cellulose Chem. Technol.*, 1972, **6**, 71.

viscose. Possible explanations for the process were discussed. Chemical modification of cellulose by thiocarbonation and redox grafting has been reported.[182] Cellulose was first allowed to react with carbon disulphide and alkali, whereafter the product was treated with either methacrylonitrile or other monomers using a peroxide-initiation system.

Cross-linked cellulose sulphate has been prepared by treating cross-linked, microcrystalline cellulose with chlorosulphonic acid in pyridine; the products (d.s. 0.1—1.1) possess cation-exchange properties.[183] The effects of reaction time, temperature, and the molar ratio of the reactants upon the degree of sulphation were investigated. The stability of hydroxypropyl cross-linkages towards chlorosulphonic acid, and the effect of sulphate groups on the solubility of the product in water were determined.

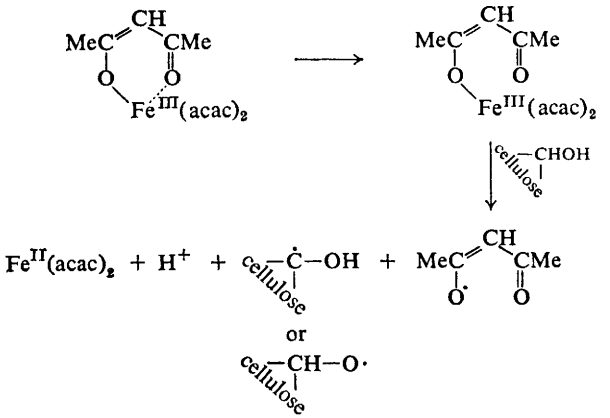
The effectiveness of ceric ammonium sulphate and nitrate as initiators of the graft polymerization of such vinyl monomers as acrylic acid, acrylonitrile, and ethyl acrylate on to cellulose has been shown to be extremely low.[184] Whereas introduction of an acid into the grafting system increased the efficiency of the initiator to a certain degree, the increase depended on the type of acid and monomer used and on the ageing of the initiator. The use of stainless steel vessels for graft polymerization of the three vinyl monomers on to cellulose, using a cerium(IV) ion initiator-system, retarded the reaction and reduced the grafting efficiency and yield.[185] The effects of varying the order of addition of the reactants, the monomer concentration, and the initiator were investigated.

Grafting on to cellulose of methyl methacrylate (emulsified in acid) has been initiated at a mercury cathode.[186] The grafting reaction took place as the stability of the emulsion was decreasing and as the homopolymer was precipitating. The grafted monomer fraction was much less than the homopolymer fraction, and systems using peroxysulphate or iron(III) ions gave inferior degrees of grafting. The location of the graft on the polysaccharide chain was briefly investigated. Determinations of the average molecular weights (by viscometry) and the polymolecularity showed that the grafted poly(methyl methacrylate) differed from the homopolymer; the molecular distribution was smaller, but the average molecular weight (2×10^6) was twenty times that of the homopolymer.[187] Two per cent of the cellulose chains appeared to have been grafted, whereas when periodate-oxidized cellulose was used, 8% of the chains were grafted. The role of aldehydes in the initiation and grafting steps of the uncatalysed polymerization of methyl methacrylate in the presence of cellulose and water has been investigated.[188] The results provide indirect evidence that added

[182] W. J. Brickman, *Tappi*, 1973, **56**, 97.
[183] J. Pastýr and L. Kuniak, *Cellulose Chem. Technol.*, 1972, **6**, 249.
[184] O. Y. Mansour and J. Schurz, *Svensk. Papperstidn.*, 1973, **76**, 415.
[185] O. Y. Mansour and J. Schurz, *Svensk. Papperstidn.*, 1973, **76**, 258.
[186] M. L. Dupraz and J. Rouger, *Cellulose Chem. Technol.*, 1973, **7**, 41.
[187] M. L. Dupraz and J. Rouger, *Cellulose Chem. Technol.*, 1973, **7**, 63.
[188] N. G. Gaylord, S. Maiti, and S. S. Dixit, *J. Polymer Sci., Part B, Polymer Letters*, 1972, **10**, 855.

aldehydes (such as glycolaldehyde or glyceraldehyde) or aldehyde–CCl_4 reaction products are significant factors in both steps when grafting is carried out in the presence of carbon tetrachloride and water.

The grafting of methyl methacrylate and styrene on to cellulose and cellulose triacetate, using ferric acetylacetonate [$Fe^{III}(acac)_3$] and manganese acetylacetonate [$Mn^{III}(acac)_3$] as initiating agents, has been studied.[189] The percentage of grafting obtained ranged from 14 to 28%, and a plausible mechanism for the initiation process involved the generation of active sites on the cellulosic substrates by a one-electron transfer-process (Scheme 19).



Scheme 19

Graft copolymers of cellulose and styrene have been prepared by heterogeneous, mutual polymerization techniques, with initiation by γ-rays.[190] The products were extracted with boiling benzene to remove attendant homopolystyrenes, whereafter the cellulose backbones were degraded by acid. The hydrolysates were separated into two fractions by t.l.c., one (A) of which contained sugar residues, due to hydrolysis at one of the chain-ends, whereas the other (B) did not. Determination of the weight fraction of the components of the hydrolysates for each graft allowed the extent of grafting to be assessed. Determinations of the number- and weight-average molecular weights (by gel-permeation chromatography) indicated that the latter values for grafted polystyrene chains are very much greater than those of polystyrene chains produced in solution. The weight-average molecular weight of fraction A (representing a true graft) was also much greater than that of fraction B, despite the fact that both were formed simultaneously within the substrate. A graft frequency of 0.03 was calculated for one of the products.

[189] B. N. Misra and C. S. Pande, *J. Polymer Sci., Polymer Chem.*, 1973, **11**, 2369.
[190] T. Taga and H. Inagaki, *Angew. makromol. Chem.*, 1973, **33**, 129.

In a report of the radiation-induced grafting of styrene on to 'high-tenacity' rayon, it was shown that conventional extraction of the homopolymer gave a value of 44% for the apparent graft.[191] However, after further extraction of the backbone- and homo-polymers, the true percentage of grafting was found to be 11%, and the graft copolymer was found to consist of one graft-branch and one backbone-chain. Calculations showed that the number of chemical bonds cleaved during irradiation was independent of the nature, fine structure, and initial degree of polymerization of the cellulose; moreover, the dose-rate and the presence of air did not significantly influence the reaction. The mechanical and tensile strengths of the products of grafting were investigated.

It has been noted that little use has been made of u.v.-irradiation in the production of graft copolymers of cellulose and vinyl monomers, since the amount of homopolymer obtained is usually considerable.[192] However, this disadvantage can be overcome by carrying out the polymerization in the vapour phase and by using a solution of ferric chloride as a photosensitizing agent. A variety of vinyl monomers could be used in this process. The interaction between the photosensitizing agent and cellulose, the modifications induced by u.v.-irradiation of the substrate, and the characterization of the grafted polymer were discussed. The effects of a number of variables on the grafting of vinyl monomers to viscose rayon, using u.v.-irradiation in the presence of solutions of ferric chloride, have also been studied.[193] The binding capacity of the cellulose substrate increased with increasing concentration of the catalyst solution and temperature, but the number of active centres was independent of the concentration of catalyst. The molecular weight of the product decreased with increasing concentration of the catalyst. The copolymer exhibited the same rheological behaviour as a physical mixture of the two homopolymers, and the apparent higher viscosity of the copolymer could be attributed to variation of the hydrodynamic volume of the macromolecules in solution. Electron microscopy confirmed the formation of a grafted polymer outside and a homopolymer inside the voids of the viscose rayon.

Cellulose has been coated with chitin by treatment with alkali-chitin; the material was shown to be stable and to possess flow-properties suitable for its use in column chromatography.[194] Chitin-coated cellulose was used in the isolation of lysozyme, which it adsorbed specifically, but which could be desorbed under mild conditions. The material has also been used for the affinity chromatography of lysozyme-like enzymes.[195]

Magnetic supports for the immobilization of enzymes have been prepared by dissolution of cellulose in a solution of cuprammonium hydroxide,

[191] I. Sakurada, T. Okada, and Y. Ikada, *Cellulose Chem. Technol.*, 1972, **6**, 35.
[192] P. Cremonesi, B. Focher, and L. D'Angiuro, *Cellulose Chem. Technol.*, 1972, **6**, 145.
[193] B. Focher, L. D'Angiuro, and P. Cremonesi, *Cellulose Chem. Technol.*, 1972, **6**, 277.
[194] T. Imoto and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 465.
[195] T. Imoto and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1191.

addition of powdered iron oxide, and extrusion of the mixture into acid.[196] After drying and breaking up into particles, the product was converted into the cyclic imidocarbonate derivative, which was coupled with β-galactosidase and chymotrypsin.

Chitin.—Colloidal chitin has been prepared by mixing the polysaccharide with cold concentrated hydrochloric acid, centrifugation of the mixture, and dilution with water to give a precipitate, which on re-suspension in water gave a colloid.[197] Colloidal chitin was used as a substrate for the assay of chitinase.

Methodology, involving partial hydrolysis with concentrated hydrochloric acid at 40 °C and separations on columns of charcoal–Celite, has been reported for the preparation of chitin oligosaccharides (*N*-acetylated).[198] Chitin oligosaccharides have also been isolated from acid hydrolysates of chitin by electrolytic desalting and gel filtration; the weight ratio of the separated mono-, di-, tri-, tetra-, penta-, and higher oligosaccharides was 15 : 6 : 6 : 6 : 6 : 7.[199] The specific preparation of penta-*N*-acetylchitopentaose, by hydrolysis of chitin with concentrated hydrochloric acid at 37 °C followed by gel filtration of the products on Bio-Gel P2, has been described. The pentasaccharide has been used as a substrate for lysozyme.[200]

Chitobiose octa-acetate, as prepared by acetolysis of chitin, is often found to contain a contaminating, acetylated amino-sugar, which is readily distinguishable from the accompanying 2-amino-2-deoxy-D-glucose penta-acetate.[201] The unknown amino-sugar has now been identified as 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5,6-di-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose. This unsaturated compound is presumed to be an artefact of the work-up procedure as a result of β-elimination undergone by an acyclic molecular species formed in the acetolysis medium.

Reduction of chitin oligosaccharides (*N*-acetylated) and gel filtration have been used to isolate penta-*N*-acetylchitopentaitol.[202] The reduced pentasaccharide was used as a substrate for β-acetamidodeoxyglucosidase.

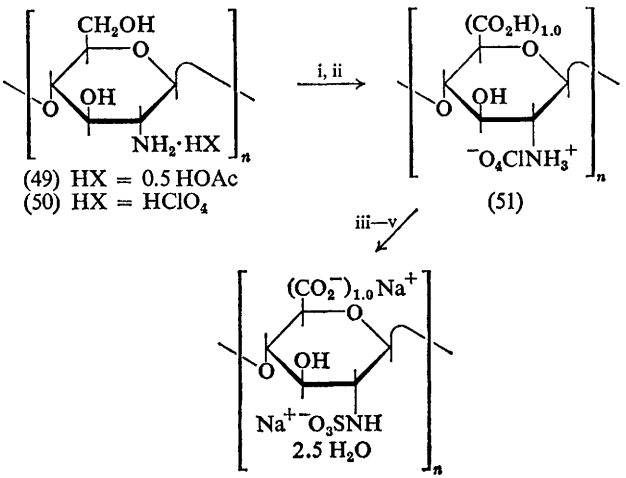
Glycol-chitin has been covalently labelled with Remazol Brilliant Blue R, and the dyed polysaccharide was used as a substrate for lysozyme.[203] After incubation with the enzyme, the remaining material of high molecular weight was precipitated, whereafter the amount of coloured material in the supernatant could be determined spectrophotometrically.

[196] P. J. Robinson, P. Dunnill, and M. D. Lilly, *Biotechnol. Bioeng.*, 1973, **15**, 603.
[197] S. Amagase, M. Mori, and S. Nakayama, *J. Biochem.* (*Japan*), 1972, **72**, 765.
[198] U. Zehavi and R. W. Jeanloz, *Biochem. Prep.*, 1971, **13**, 14.
[199] R. C. W. Berkeley, S. J. Brewer, and J. M. Ortiz, *Analyt. Biochem.*, 1972, **46**, 687.
[200] J.-P. Périn and P. Jollès, *Clinica Chim. Acta*, 1972, **42**, 77.
[201] E. W. Thomas, *Carbohydrate Res.*, 1973, **26**, 225.
[202] T. Mega, T. Ikenaka, H. Arita, K. Fukukawa, and Y. Matsushima, *J. Biochem.* (*Japan*), 1973, **73**, 55.
[203] N. Yamasaki, T. Tsujita, and M. Takakuwa, *Agric. and Biol. Chem.* (*Japan*), 1973, **37**, 1507.

Titanium chelates of chitin, in which at least one of the water ligands of the hexaco-ordinated titanium ions is replaced by a polysaccharide hydroxy-group, have been prepared.[102] The chitin chelate was used for the immobilization of glucose oxidase, with retention of enzymic activity. The immobilization process is considered to involve the replacement of a water ligand of the bound titanium ion by an amino- or carboxy-group of the enzyme.

Chitin-coated cellulose has been prepared by treatment of cellulose with alkali chitin (see p. 547), and was used for the affinity chromatography of lysozyme [194] and certain lysozyme-like enzymes.[195]

Chitosan has been converted *via* the acetate salt (49) into the perchlorate salt (50) (Scheme 20).[204] The latter salt underwent complete and specific



(49) HX = 0.5 HOAc
(50) HX = $HClO_4$

(51)

(52)

Reagents: i, $HClO_4$; ii, CrO_3; iii, C_5H_5N; iv, $ClSO_3H$–C_5H_5N; v, 1M-NaOH

Scheme 20

oxidation at C-6 with chromic oxide to give the (1 → 4)-linked 2-amino-2-deoxy-β-D-glucopyranuronan (51). *N*-Sulphation (with chlorosulphonic acid) then afforded a (1 → 4)-linked 2-deoxy-2-sulphoamino-β-D-gluco-pyranuronan, which was isolated as the amorphous sodium salt (52). The latter compound displayed moderate blood-anticoagulant activity, and both (51) and (52) markedly inhibited the growth of leukaemia L-1210 cells *in vitro*.

Cycloamyloses.—The preparation of *trans*-2,3-cyclic carbonate derivatives of cyclohexa- and cyclohepta-amyloses, by treatment of the oligosaccharides with ethyl chloroformate, has been described.[99] Special extraction

[204] D. Horton and E. K. Just, *Carbohydrate Res.*, 1973, **29**, 173.

procedures were necessary for the isolation of the cyclic carbonates, since very high losses occurred when the work-up procedure used with cellulose *trans*-2,3-cyclic carbonate was applied. The presence of the *trans*-2,3-cyclic carbonate groups, and also of a proportion of acyclic carbonate (ethyloxycarbonyl) groups, in the products was revealed by i.r. spectroscopy. From these and similar reactions with other polysaccharides, it appeared that the relative proportion of cyclic and acyclic groups is a function of the reaction conditions rather than the availability of free hydroxy-groups at C-6. Possible applications of the cycloamylose derivatives in the field of immobilization were discussed.

Comparisons have been made of the i.r. spectra of cyclohexa-amylose, cyclohepta-amylose, and other polysaccharides that had been nitrated in various ways.[27] The use of i.r. spectroscopy for quantification of the nitrate groups in the products was discussed.

Studies to elucidate the nature of the crystalline complexes formed from cycloamyloses and solutions of guest molecules in diethyl ether have been reported.[205] *X*-Ray crystallographic and other data on complexes of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses with the guest molecules 1-naphthylamine, azobenzene, anthracene, and 8-hydroxyquinoline indicated that diethyl ether and guest molecules are both included within the voids of the cyclodextrins and play an important role in forming the channel-like structures of these molecules. The effects of inclusion of guest molecules on the crystalline structures of the cycloamyloses were investigated.

Cyclohepta-amylose has been examined as a potentially useful probe of the structures of nucleic acids.[206] The binding constant and the enthalpy and entropy for the binding of adenosine 5′-phosphate were determined, and the c.d. spectrum of the complex was calculated. The binding of adenosine 5′-phosphate, adenosine 3′,5′-cyclic phosphate, adenosine, and adenine were also examined. No evidence was obtained for the involvement of hydrophobic forces in the binding, and c.d. data for the products resulting from treatment of cyclohepta-amylose with the 5′-phosphates of guanosine, uridine, and cytidine revealed that the binding of nucleotides is not as specific as previously reported.

A synthetic model of an enzyme, based on a cycloamylose and catalysing the hydrolyses of synthetic esters, has been described.[207] The hollow arising in the structure of the cycloamylose molecule is considered to replicate the spatially active site of the enzyme. Introduction of nickel chelated with pyridinecarboxadoxime (as an artificial catalytic group) into this site produced a very active model hydrolase, the basic macromolecular skeleton being a polysaccharide instead of the protein of natural enzymes.

The inclusion of 2-hydroxymethyl-4-nitrophenyl trimethylacetate within the cavity of cyclohexa-amylose produced a six-fold acceleration in its rate

[205] K. Takeo and T. Kuge, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2615.
[206] C. Formoso, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 999.
[207] V. Kaláč and K. Babor, *Chem. listy*, 1972, **66**, 1299.

of conversion into 2-hydroxy-5-nitrobenzyl trimethylacetate.[208] By contrast, formation of the inclusion complex with cyclohepta-amylose decelerated the rate of intramolecular transesterification by a factor of five. It was suggested that these cycloamyloses, by virtue of their ability to include organic molecules within rigid binding-sites, perturb the equilibrium between the orientational conformers of 2-hydroxymethyl-4-nitrophenyl trimethylacetate, thereby forcing the reacting groups to assume mutually favourable (or unfavourable) orientations with respect to the activated complex. It was also concluded that binding forces between the cycloamyloses and 2-hydroxymethyl-4-nitrophenyl trimethylacetate are responsible for these orientational restrictions.

Dextrans.—A series of end-labelled isomalto-oligosaccharides has been prepared by the reaction of dextransucrase with ^{14}C-labelled sucrose in the presence of an excess of unlabelled isomalto-oligosaccharides as alternative acceptors.[209] The main product of each reaction contained one more D-glucose residue than the acceptor substrate, with the label at the non-reducing end. The labelled oligosaccharides were used to determine the substrate specificity of a dextranase.

Isomalto-oligosaccharides have been oxidized to the corresponding aldonic acids, and the modified oligosaccharides were coupled to protein using isobutyl chloroformate in aqueous DMF; the linkage to the oligosaccharides is considered to involve lysyl residues of the protein.[17] The products were used as carbohydrate antigens.

The reactions of dextran cyclic carbonate with amino-acids and their derivatives, polypeptides, and human serum albumin have been studied.[210] The degrees of coupling achieved were compared for both water-soluble and -insoluble forms of the dextran carbonate.

The i.r. spectra of variously nitrated dextran and other polysaccharides have been compared, and the use of i.r. spectroscopy to quantify the extent of nitration was assessed.[27]

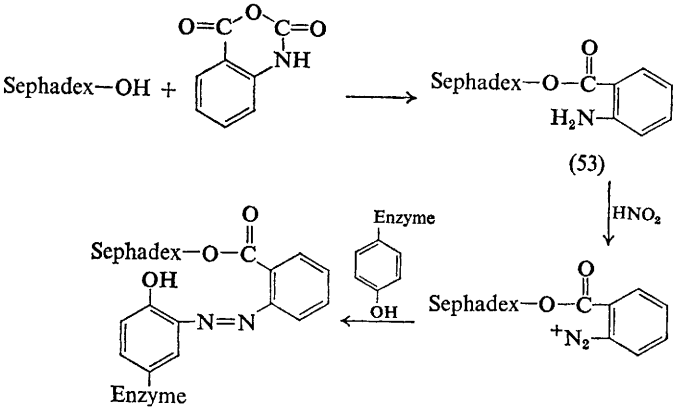
Dextran and materials of lower molecular weight obtained by mild acid hydrolysis of the polysaccharide have been sulphated using either concentrated sulphuric acid or chlorosulphonic acid.[211] The abilities of the sulphated products, and similar products derived from laminarin and xylan, to liberate diacylglycerol lipase into the blood, as well as the inhibition of this activity, were investigated. It was found that the activities and the inhibition thereof are proportional.

The influence of intravenous injection of a complexed iron–dextran preparation (Imferon) on β-glucuronidase activities in rabbits has been studied.[212]

[208] D. W. Griffiths and M. L. Bender, *J. Amer. Chem. Soc.*, 1973, **95**, 1679.
[209] G. J. Walker, *Carbohydrate Res.*, 1973, **30**, 1.
[210] S. A. Barker, H. M. Disney, and P. J. Somers, *Carbohydrate Res.*, 1972, **25**, 237.
[211] S. Yasuoka, S. Fujii, T. Yamaguchi, K. Sakurai, and T. Okuyama, *J. Biochem.* (*Japan*), 1973, **74**, 425.
[212] B. Ballantyne and P. J. Guillou, *Microbios*, 1973, **7**, 181.

A book on the properties and applications of ion-exchangers has covered their uses as preparative agents in industry, analytical chemistry, pharmacy, and medicine, and includes sections on Sephadex (a commercially available epichlorohydrin-cross-linked dextran) derivatives.[115]

The reaction of Sephadex with isatoic anhydride to give anthranilate esters (53) has been reported (Scheme 21).[213] Diazotization of these



Scheme 21

derivatives gave matrices suitable for the covalent attachment of such enzymes as trypsin and subtilisin.

DEAE-Sephadex has been employed as a matrix for the immobilization of aspartate ammonia-lyase, the bond between the two molecules being ionic.[139] The immobilized enzyme was used in the production of L-aspartic acid by a continuous process.

Sephadex cyclic imidocarbonate reacted with aminopeptidase (cytosol) that had been labelled with fluorescein isothiocyanate to provide a means of visualizing the bound protein.[31] Alternatively, the bound protein was visualized by allowing Sephadex cyclic imidocarbonate to react with the underivatized enzyme and then treating the immobilized enzyme with homologous antibody labelled with fluorescein isothiocyanate.

Sephadex coupled to anti-insulin has been used in an immunoassay of insulin by fluorometry of an insulin–glucoamylase complex.[214]

Calcium ions have been shown to bind weakly to Sephadex.[122]

Eremuran.—Precipitation of the neutral polysaccharides eremuran (a glucomannan), glycogen, inulin, and starch, and the separation of mixtures thereof using various quaternary salts have been reported.[215] The polysaccharides were complexed with borate ions, to render them acidic, before

[213] N. E. Franks, *Biotechnol. Bioeng.*, 1972, **14**, 1027.
[214] E. Ishikawa, *J. Biochem. (Japan)*, 1973, **73**, 1319.
[215] B. N. Stepanenko and L. B. Uzdenikova, *Carbohydrate Res.*, 1972, **25**, 526.

treatment with the quaternary salts. In a parallel study in which the salts of nine quaternary bases and the same polysaccharides were employed, it was shown that mixtures of starch and eremuran could be fractionated.[216] Trimethyloctylammonium bromide (octalon) was found to be most suitable for this separation, since it precipitated starch and left eremuran in solution.

Glycogens.—The influence of treatment of glycogen with alkali on its chromatographic behaviour has been investigated.[217]

The precipitation of a number of neutral polysaccharides (including glycogen) and the separation of mixtures thereof using quaternary salts have been reported (see p. 552).[215, 216] It was also found that dimethyldodecylbenzylammonium chloride was more efficient than Cetavlon in the fractionation of glycogen and inulin. The precipitation of β-limit dextrins of glycogen by salts of quaternary bases has also been examined.[104]

Glycosaminoglycuronans and Glycosaminoglycans.—The conformations of chondroitin sulphate, dermatan sulphate, heparin, and keratan sulphate in solution have been deduced from the induced Cotton effects of their Methylene Blue complexes.[113] The results were compared with those obtained for λ- and κ-carrageenans, and it was concluded that they reveal general patterns of conformational preference among these polysaccharides and the importance of substituent groups in maintaining the conformation.

The interactions between chondroitin 6-sulphate and poly(L-lysine) have been studied as a model for possible complex formation between fibrous proteins and glycosaminoglycans.[218] Results derived from the c.d. spectra showed that the conformation-directing action of chondroitin 6-sulphate upon the poly(amino-acid) is maximal when the ratio of L-lysyl to disaccharide residues is 1 : 1. Changes occurring in the c.d. spectrum of the 1 : 1 mixture, following either an increase in ionic strength or the addition of non-polar solvents, indicated that the interaction between the polysaccharide and poly(L-lysine) is ionic in nature. Such effects were not observed for non-sulphated glycosaminoglycans admixed with poly(L-lysine), suggesting that the sulphate groups, rather than the carboxy-groups, of chondroitin 6-sulphate interact with the amino-groups of the poly(amino-acid). Elevation of the temperature above normal disrupted these interactions.

Hyaluronic acid–Acridine Orange complexes in water showed a symmetrical doublet centred at 455 cm^{-1} in the c.d. spectra.[219] The exciton-like c.d. band indicated that left-handed chirality is preferred for the polymer in solution, and so correlates with the proposed left-handed, double-helical structure of hyaluronic acid in oriented films. The sign and general shape of the induced c.d. band remained the same throughout the range of

[216] B. N. Stepanenko and L. B. Uzdenikova, *Biokhimiya*, 1973, **38**, 52.
[217] L. N. Bobrova and B. N. Stepanenko, *Doklady Akad. Nauk S.S.S.R.*, 1973, **209**, 980.
[218] R. A. Gelman, W. B. Rippon, and J. Blackwell, *Biopolymers*, 1973, **12**, 541.
[219] B. Chakrabarti and E. A. Balazs, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 1170.

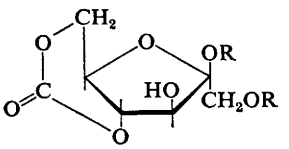
polymer : dye ratios studied. Hyaluronic acid that had been pre-heated to 95 °C and rapidly cooled did not show an induced c.d. band.

A 9.5*S* α_1-glycoprotein from human serum possessing metal-binding properties was found to have affinity for, and to form a complex with, heparin.[32]

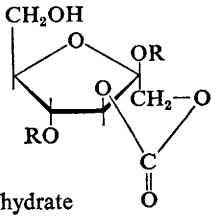
[^{35}S]Sulphate-labelled oligosaccharides have been prepared from heparan [^{35}S]sulphate, labelled *in vitro*, by degradation with nitrous acid.[220]

A glycopeptide derived from bovine nasal septum by sequential treatments with trypsin, chymotrypsin, methanolic hydrogen chloride (desulphation), hyaluronidase, and β-glucuronidase has been converted into an immobilized form by reaction with agarose cyclic imidocarbonate.[75] The product was used for the partial purification of β-acetamidodeoxyhexosidase by affinity chromatography.

Inulin.—Inulin reacted with ethyl chloroformate to form a carbonate derivative.[99] The occurrence of a carbonyl band in the i.r. spectrum of the inulin carbonate at 1820 cm^{-1}, in addition to a band at 1750 cm^{-1} corresponding to acyclic carbonate (*O*-ethoxycarbonyl groups), was attributed to the formation of strained *trans*-4,6-cyclic carbonate groups on the D-fructofuranosyl residues (54), whereas *trans*-2,3-cyclic carbonate groups are presumably formed with the relatively small number of terminal



(54)



R = adjacent carbohydrate residues

(55)

D-glucopyranosyl residues. In addition to formation of *trans*-4,6-cyclic carbonate groups on terminal D-fructofuranosyl residues, *trans*-1,3-cyclic carbonates (55) might also be formed, since this ring has the same stereochemistry as the *trans*-4,6-cyclic carbonate. From similar derivatizations of a number of polysaccharides and cycloamyloses, it appeared that the relative proportions of cyclic and acyclic groups is a function of the reaction conditions rather than the availability of free hydroxy-groups at C-6. Potential applications of the inulin derivative in the field of immobilization were discussed.

Precipitation of the neutral polysaccharides inulin, starch, eremuran, and glycogen from aqueous solutions, and the separation of mixtures thereof using various quaternary salts have been reported (see p. 552).[215, 216] It was found that dimethyldodecylbenzylammonium chloride is more

[220] H. Kresse, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 1111.

efficient than Cetavlon in the fractionation of mixtures of inulin and glycogen.

Laminarin.—The oxidation of laminarin with periodate ion has been reported, and the product was used as a substrate in studies of β-glucanases.[221]

Laminarin has been sulphated using either concentrated sulphuric acid or chlorosulphonic acid.[211] Sulphated materials of low molecular weight were obtained by mild acid hydrolysis of laminarin prior to sulphation. The abilities of sulphated laminarin and other sulphated polysaccharides to liberate diacylglycerol lipase into the blood, and inhibition of this enzymic activity, were investigated; it was found that the abilities of the sulphated polysaccharides to liberate and to inhibit the enzyme are proportional.

Levans.—Carbohydrate residues were added to acceptor levans in the transfructosylation reaction catalysed by levansucrase.[7]

Mannans.—Carboxymethylation of ivory nut (*Phytelephas macrocarpa*) mannan using chloroacetic acid and alkali has been described.[222] The carboxymethylmannans (d.s. 0.4—0.7) obtained are soluble in water, giving solutions of low viscosity.

Yeast mannan has been insolubilized by entrapment in a polyacrylamide gel, and the modified form was used for immunoadsorption of antibodies to the mannan.[223]

Nigerans.—The preparation of a water-insoluble *trans*-2,3-cyclic carbonate of nigeran has been described, and the presence of cyclic and acyclic (ethyloxycarbonyl) carbonate groups in the product was revealed by i.r. spectroscopy.[99] From similar reactions with other polysaccharides and cycloamyloses, it appeared that the relative contents of cyclic and acyclic carbonate groups are a function of the reaction conditions rather than the availability of free hydroxy-groups at C-6. Potential applications of the nigeran derivative in the field of immobilization were discussed.

Pectic Acids.—^{14}C-Labelled pectic acid has been prepared from *Chara globate* by growth of the plant in the presence of [^{14}C]carbon dioxide.[224] The polysaccharide was shown to be uniformly labelled, and labelled oligosaccharides were obtained from it by partial hydrolysis with polygalacturonase.

It has been shown that depolymerization of pectin with alkali occurs much faster, and is less dependent on the concentration of alkali, than in the case of hydroxypropylalginate (see p. 526).[100]

The rapid decrease in viscosity of solutions of polygalacturonic acid occurring in the presence of periodate ions has been compared with the behaviours of alginic acid, methylated cellulose, and non-carbohydrate

[221] V. Farkaš, P. Biely, and Š. Bauer, *Biochim. Biophys. Acta*, 1973, **321**, 246.
[222] N. Desveaux and F. Percheron, *Compt. rend.*, 1973, **276**, *C*, 355.
[223] H. Gerber, M. Horisberger, and H. Bauer, *Infection and Immunity*, 1973, **7**, 487.
[224] Y. Milner and G. Avigad, *Carbohydrate Res.*, 1972, **25**, 153.

polymers under identical conditions.[25] A mechanism for the cleavage, based on known disproportionations of ether-type free radicals, has been proposed (see Scheme 13).

The reaction of pectic acid with tris(diethylamino)phosphine [$P(NEt_2)_3$] or dipropyl *NN*-diethylphosphoramidite [$(n\text{-}C_3H_7O)_2PNEt_2$] gave *NN*-dialkylamides having various degrees of substitution.[225] Owing to the insoluble nature of pectic acid, the polysaccharide was pre-treated with a mixture of diethylamine, pyridine, DMSO, and DMF. Conditions affecting the degree of substitution of the products were investigated. The product galacturonan amides are amorphous, soluble in water, and unstable to acids.

Starch.—An article on the present, future, and industrial uses of carbohydrates includes a section on starch derivatives.[114]

Uniformly ^{14}C-labelled starch has been used for studies of the substrate specificities of certain α-glucosidases and for distinguishing between the maltose- and starch-hydrolysing sites of the enzymes.[226]

The conversion of starch and starch dextrin into a polysaccharide containing D-glucose, D-galactose, D-mannose, and uronic acid (probably D-glucuronic acid) by cultures of *Achromobacter mucosum* has been described.[227]

Oxidation of starch by hydrogen peroxide in the presence of u.v. light furnished a product containing significant proportions of carbonyl and carboxy-groups.[228] The use of such reagents as ferrous sulphate or sodium hypochlorite in the oxidation system did not increase the proportions of the various types of groups formed.

When rats were fed with diets containing hydroxypropylated starches (d.s. $\leqslant 0.11$), the major metabolite isolated from the faeces was shown (by mass spectrometry and ^1H n.m.r. spectroscopy of its fully acetylated derivative) to be 4-*O*-[2-*O*-(*RS*)(2-hydroxypropyl)-α-D-glucopyranosyl]-D-glucopyranose.[229] 4-*O*-[6-*O*-(*RS*)(2-Hydroxypropyl)-α-D-glucopyranosyl]-D-glucose has also been identified as a faecal metabolite of hydroxypropylated starches in rats.[230]

A comparison has been made of the i.r. spectra of starch and other polysaccharides that had been nitrated in various ways, and the use of i.r. spectroscopy for quantitative determination of nitrate groups in starch nitrate was discussed.[27]

Two samples of tapioca starch having different viscosities in aqueous solution have been cross-linked with sodium trimetaphosphate, epichloro-

[225] V. P. Evdakov, I. M. Khorlina, and N. M. Khelemskaya, *Zhur. obshchei Khim.*, 1973, **43**, 388.
[226] N. Takahashi and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 67.
[227] Y. Ozawa, K. Yamada, H. Kobayashi, and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2117.
[228] R. E. Harmon, S. K. Gupta, and J. Johnson, *Stärke*, 1971, **23**, 347.
[229] D. C. Leegwater, M. C. ten Noever de Brauw, A. Mackor, and J. W. Marsman, *Carbohydrate Res.*, 1972, **25**, 411.
[230] D. C. Leegwater and J. W. Marsman, *Carbohydrate Res.*, 1973, **29**, 271.

hydrin, and phosphorus oxychloride; epichlorohydrin was found to be the most efficient of these reagents.[231] It was noted that the sample having the higher viscosity required less of the cross-linking agent for stabilizing the viscosity of its paste.

A recently published book contains sections dealing with the synthesis of graft and block copolymers of starch, as well as with the properties and applications of these materials.[232]

Graft copolymers and terpolymers of starch and vinyl monomers (acrylamide, acrylic acid, acrylonitrile, and β-methacryloyloxyethyltrimethylammonium monomethyl sulphate) have been prepared and evaluated as flocculants.[233] Initiation was carried out by γ-irradiation, except for the copolymerization with acrylonitrile that was initiated by cerium(IV) ions. The copolymer with acrylonitrile was hydrolysed with base to a terpolymer of acrylamide and acrylic acid before testing. The graft copolymer of starch and acrylic acid proved to be an effective flocculant for red-mud suspensions of bauxite ore.

The graft copolymerization of acrylonitrile to starch using ceric ammonium nitrate as the initiator has been further studied.[234] The polyacrylonitrile chains within the starch granules were located after hydrolysis of the polysaccharide with hot, dilute hydrochloric acid; areas in the grafted granules containing little or no polyacrylonitrile appeared as voids in the fracture surfaces revealed by electron microscopy. Data from the electron micrographs suggested that granules of low add-on graft copolymer were largely surface-grafted, whereas grafting had taken place throughout most of the interior of the granule in the higher add-on product.

Copolymers of potato starch grafted with either poly(acrylic acid) or poly(methacrylic acid) by the Scott-xanthation process have been shown to consist of a backbone of amylopectin to which is attached side-chains of the synthetic polymer; it was found that there are always twice as many units of the side-chain polymer as D-glucose units in the graft copolymer.[235] The molecules were spherical and exhibited a relatively high coil-density in solution. It was suggested that amylopectin serves as a matrix for the propagation of the grafting reaction in which the side-chains are lined up along the backbone and attached by weak bonds.

Precipitation of the neutral polysaccharides starch, inulin, glycogen, and eremuran from aqueous solutions, and the separation of mixtures thereof using various quaternary ammonium salts have been reported (see p. 552).[215, 216] Trimethyloctylammonium bromide (octalon) was particularly effective in these separations, starch being precipitated and eremuran or glycogen remaining in solution.

[231] H. C. Srivastava and M. M. Patel, *Stärke*, 1973, **25**, 17.
[232] R. J. Ceresa, 'Block and Graft Copolymerization', Wiley, Chichester, 1973.
[233] D. A. Jones and L. F. Elmquist, *Stärke*, 1973, **25**, 83.
[234] G. F. Fanta, F. L. Baker, R. C. Burr, W. M. Doane, and C. R. Russell, *Stärke*, 1973, **25**, 157.
[235] E. Gruber, S. Alloush, and J. Schurz, *Stärke*, 1973, **25**, 39.

Xylans.—The preparation of a water-insoluble carbonate derivative on treatment of xylan with ethyl chloroformate has been described.[99] The presence of *trans*-2,3-cyclic and acyclic (ethyloxycarbonyl) carbonate groups in the product was revealed by i.r. spectroscopy. Possible applications of the xylan derivative in the field of immobilization were discussed.

In a study of the thermal degradation of xylan and related model compounds, the behaviours of 4-*O*-methylglucuronoxylan and an acetylated derivative thereof were investigated.[236] Thermal degradation was found to involve cleavage of the glycosidic bonds to give a number of products, several of which were identified.

A β-(1 → 4)-linked xylan and materials of low molecular weight derived from it by mild acid hydrolysis have been sulphated using either concentrated sulphuric acid or chlorosulphonic acid.[211] The abilities of the sulphated polysaccharide to liberate diacylglycerol lipase into the blood, and the inhibition of the activity of this enzyme were investigated. For the xylan and other polysaccharide sulphates similarly obtained, these activities and the inhibition thereof are proportional.

Miscellaneous Polysaccharides.—Fourteen pneumococcal polysaccharide types (I—IX inclusive, XII, XIV, XVIII, XIX, and XXIII) have been individually coated on to red-blood cells using chromic chloride as the coupling agent.[237] The cells were treated with the polysaccharide prior to the addition of chromic chloride, and optimal conditions for coating were determined. The coupled products were used for the quantification of serum antibodies to type-specific pneumococcal polysaccharides, and it was suggested that they could be used to determine the response to antibodies following immunizations of large populations.

A succinoglycan (containing residues of D-galactose, D-glucose, and succinic acid) obtained from *Alcaligenes faecalis* has been desuccinylated using dilute alkali at 70 °C.[238]

Cells of *Micrococcus lysodeikticus* have been modified by *N*-acetylation and partial *O*-methylation.[239] After extraction with hot hydrazine, the product was used as a substrate for studies with lysozyme.

Modification of Glycoproteins and Uses of Modified Glycoproteins

Standard methodology for tritium-labelling of glycoproteins containing either terminal D-galactose [240] or terminal sialic acid residues [241] has been

[236] F. Shafizadeh, G. D. McGinnis, and C. W. Philpot, *Carbohydrate Res.*, 1972, **25**, 23.
[237] A. J. Ammann and R. J. Pegler, *Appl. Microbiol.*, 1972, **24**, 679.
[238] T. Harada, K. Moori, and A. Amemura, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2611.
[239] T. Monodane, S. Hara, and Y. Matsushima, *J. Biochem. (Japan)*, 1972, **72**, 1175.
[240] A. G. Morell and G. Ashwell in 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Vol. XXVIII, ed. V. Ginsburg, Academic Press, New York, 1972, p. 205.
[241] L. Van Lenten and G. Ashwell in 'Methods in Enzymology', ed. S. P. Colowick and N. O. Kaplan, Vol. XXVIII, ed. V. Ginsburg, Academic Press, New York, 1972, p. 209.

described. Terminal D-galactose residues were exposed, where necessary, by desialylation of the glycoprotein with sialidase, after which they were oxidized at C-6 with galactose oxidase and reduced with sodium borotritide. Labelling of terminal sialic acid residues was accomplished by oxidation with periodate ion, followed by reduction with sodium borotritide.

Concanavalin A has been converted into a 2-hydroxy-5-nitrobenzyl derivative by reaction with 2-bromomethyl-4-nitrophenol in alkali, and the derivative was used to study carbohydrate-induced changes in the conformation of the phytohaemagglutinin.[242] Treatment of concanavalin A with fluorescein isothiocyanate gave a fluorescein-labelled derivative, which was used to reveal the site of mannan insertion in replication of the cell wall in a *Saccharomyces* species.[243] Interaction of concanavalin A with arylsulphatase has been found to precipitate a complex, and, since the enzyme was still active in this water-insoluble form, it could be used as an immobilized form of the enzyme.[244]

Collagen modified in various ways has been used as a support for the immobilization of enzymes. A collagen dispersion mixed with β-fructofuranosidase was cast on to a nylon support to form a membrane, which was then tanned with glutaraldehyde.[245] Alternative methods involved the impregnation of a pre-swollen collagen membrane with β-fructofuranosidase, lysozyme, urease, or penicillin amidase, or the electrodeposition of the complex from a collagen dispersion containing dissolved glucose oxidase or glucose isomerase.[246] The mechanism of complex formation between collagen and the enzymes involves multiple ionic linkages, hydrogen bonds, and van der Waals interactions. An analogous use of collagen involved the binding of heat-treated cells of *Streptomyces phaeochromogenes* containing active glucose isomerase, followed by tanning of the collagen with either alkaline formaldehyde or glutaraldehyde to the desired mechanical strength to produce an active immobilized form of the enzyme.[247]

The lability of human pituitary follicle-stimulating hormone to dilute hydrochloric acid has been studied, and the removal of *N*-acetylneuraminic acid residues by acidic and enzymic hydrolyses has been compared.[248] Hydrolysis at pH 1 and 20 °C for 3 hours abolished the biological activity of the hormone *in vivo*, but similar hydrolysis at pH zero and 20 °C liberated fragments of low molecular weight that retained the immunological activity. The mildest condition needed to remove all the *N*-acetylneuraminic acid residues was pH 2 at 80 °C for 50 minutes. Under these conditions, 75%

[242] R. J. Doyle, S. K. Nicholson, R. D. Gray, and R. H. Glew, *Carbohydrate Res.*, 1973, **29**, 265.
[243] J. S. Tkacz and J. O Lampen, *J. Gen. Microbiol.*, 1972, **72**, 243.
[244] A. Ahmad, S. Bishayee, and B. K. Bachhawat, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 730.
[245] K. Venkatasubramanian and W. R. Vieth, *Biotechnol. Bioeng.*, 1973, **15**, 583.
[246] S. S. Wang and W. R. Vieth, *Biotechnol. Bioeng.*, 1973, **15**, 93.
[247] W. R. Vieth, S. S. Wang, and R. Saini, *Biotechnol. Bioeng.*, 1973, **15**, 565.
[248] J. F. Kennedy and M. F. Chaplin, *J. Endocrinol.*, 1973, **57**, 501.

of the L-fucosyl residues, but none of the other monosaccharide residues, of the hormone were liberated. The results provide an example in which acid hydrolysis is unsuitable for the selective removal of *N*-acetylneuraminic acid and L-fucose residues from a glycoprotein. On the other hand, selective removal of the *N*-acetylneuraminic acid residues with neuraminidase was satisfactorily achieved.

The reactions of bovine pituitary luteinizing and thyroid-stimulating hormones with tetranitromethane have been compared.[249] When large amounts of the reagent were employed, the total reaction products were inactive as hormones. Although polymer formation was predominant, a nitrated monomer of luteinizing hormone was obtained that could be separated into α- and β-subunits and a fraction containing cross-linked monomer. The nitrated α-subunit of the luteinizing hormone was able to recombine with the β-subunits of both luteinizing and thyroid-stimulating hormones, and the immunological response of luteinizing hormone was unimpaired by nitration. In the case of intact thyroid-stimulating hormone, nitration with even a limited amount of reagent yielded largely polymeric material (molecular weight $1.5—2.2 \times 10^5$), but the immunological activity was unaffected.

Human α_1-acid glycoprotein and bovine fetuin have been tritiated by reductive methylation of the ε-amino-group of lysyl residues with formaldehyde and borotritide ion.[250] Molecular-sieve chromatography of the labelled glycoproteins showed that derivatization did not significantly affect the molecular size. The tritiated glycoproteins exhibited plasma half-lives in rats similar to those obtained when the glycoproteins were labelled in other ways. Reductive methylation did not impair the ability of the glycoproteins to disappear promptly from circulation after desialylation.

4-Azophenobarbital derivatives of acetylated bovine serum albumin, rabbit serum albumin, bovine immunoglobulin, and egg albumin have been obtained by treating the macromolecules with diazotized 4-aminophenobarbital.[251] The derivative obtained from bovine serum albumin was used to produce anti-phenobarbital antibodies in rabbits.

Asialo-fetuin has been prepared by hydrolysis of fetuin at 80 °C for 1 hour in 0.06N-sulphuric acid, whereafter D-galactose was removed by Smith degradation.[252] The asialo-agalacto-fetuin was able to inhibit the binding of ^{125}I-labelled asialo-fetuin by plasma-membrane fragments of rat liver. Since the asialo-agalacto-derivative is not a substrate for plasma-membrane sialyltransferase, it is considered to be a true inhibitor of the membrane-binding reaction.

Glycopeptides obtained from desialylated bovine fibrinogen and *N*-terminal-acetylated bovine fibrinogen reacted with Sepharose cyclic

[249] K. Cheng and J. G. Pierce, *J. Biol. Chem.*, 1972, **247**, 7163.
[250] G. Gregoriadis and B. E. Ryman, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 1134.
[251] H. Satoh, Y. Kuroiwa, and A. Hamada, *J. Biochem. (Japan)*, 1973, **73**, 1115.
[252] N. N. Aronson, L. Y. Tan, and B. P. Peters, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 112.

imidocarbonate to form affinity-chromatography matrices.[77] Following the acetylation reaction, subsequent derivatization with trinitrobenzenesulphonic acid indicated that only 4% of the original amino-groups remained unacetylated.

[^{14}C]Bicarbonate-labelled transferrin has been prepared in order to study the role of bicarbonate in the cell-mediated release of iron from transferrin.[253] [^{14}C]Bicarbonate ions became bound to transferrin only in the presence of iron, with a ratio of bound bicarbonate to bound iron of unity. The transferrin–bicarbonate complex was very stable over the pH range 7.5—9.0, even in the presence of unbound bicarbonate, but exchange between labelled and unlabelled bicarbonate ions was promoted by oxalate, citrate, and phosphate ions.

In a study of the cross-linking of bovine erythrocyte membrane with glutaraldehyde, it appeared that all constituents, with the exception of glycoproteins, became cross-linked.[254]

Affinity labelling of the active sites of antibodies and myeloma 'proteins' by reaction with 4-diazophenylarsonic acid, 4-nitrophenyldiazonium and 3-nitrophenyldiazonium cations, and trimethylamino-4-diazophenyl dications has been described.[255] Antibodies to β-fructofuranosidase have been labelled with fluorescein, and the modified form was used to ascertain the surface distribution of the enzyme on growing cells of a *Saccharomyces* species.[256] Antibodies to aminopeptidase (cytosol) have been labelled with fluorescein isothiocyanate and were used for detection of the enzyme following its reaction with Sepharose cyclic imidocarbonate.[31]

Preparations of many immobilized, insolubilized, and coupled glycoproteins have been described, and these derivatives, together with their applications, are summarized in Table 6.[257, 258] Monosaccharides can be fractionated by elution from a column of concanavalin A immobilized on agarose cyclic imidocarbonate using aqueous phosphate buffer at nearly neutral pH as eluant.[56, 57] Branched-chain polysaccharides can also be fractionated on this column. Weakly interacting polysaccharide fractions were eluted with phosphate buffer, whereas tightly bound fractions were eluted with borate buffer; the use of borate buffer overcame problems arising from elution of the bound polysaccharide with other sugars. The work also demonstrated that mixtures of carbohydrates, which are not separated by complex formation with concanavalin A in solution, can be separated using an immobilized form of the lectin.

In a study of immunological reactions carried out at a liquid–solid interface, antigens have been immobilized by reaction with nickel-plated

[253] J. Martinez-Medellin and H. M. Schulman, *Biochem. Biophys. Res. Comm.*, 1973, **53**, 32.
[254] R. A. Capaldi, *Biochem. Biophys. Res. Comm.*, 1973, **50**, 656.
[255] S. J. Singer, N. Martin, and N. O. Thorpe, *Ann. New York Acad. Sci.*, 1971, **190**, 342.
[256] J. C. Tkacz and J. O. Lampen, *J. Bacteriol.*, 1973, **113**, 1073.
[257] A. Rothen, *Biophys. J.*, 1973, **13**, 402.
[258] D. Zabriskie, D. F. Ollis, and M. M. Burger, *Biotechnol. Bioeng.*, 1973, **15**, 981.

Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule of matrix coupled*	*Use of product*	*Ref.*
Albumin	Agarose cyclic imidocarbonate	Production of immobilized gangliosides	61
Albumin (urea-denatured)	Agarose cyclic imidocarbonate	Production of immobilized gangliosides	61
Anti-bovine serum albumin	Bovine serum albumin on activated nickel-plated slides	Study of immunological reactions carried out at a liquid–solid interface	257
Anti-deoxyribonuclease (immunoglobulin IgG)	Agarose cyclic imidocarbonate	Isolation of deoxyribonuclease by affinity chromatography	41
Anti-2,4-dinitrophenyl	Agarose cyclic imidocarbonate	Isolation of trypsin by modified affinity chromatography in which trypsin is complexed with 2,4-dinitrophenyl–soybean trypsin inhibitor before chromatography	38
Anti-human follicle-stimulating hormone	Cellulose *trans*-2,3-cyclic carbonate	Measurement of human follicle-stimulating hormone by radioimmunoassay	146
Anti-human μ-immunoglobulin light-chains	Agarose cyclic imidocarbonate	Removal of immunoglobulin IgM from normal human sera	39
Anti-insulin	Sephadex derivative	Measurement of insulin by immunoassay with glucoamylase	214
Bovine fibrinogen glycopeptide	Agarose cyclic imidocarbonate	Demonstration of preparation of affinity-chromatography materials using glycopeptides	77
Bovine fibrinogen glycopeptide (*N*-acetylated)			
Calf corneal glycoprotein glycopeptide			
Collagen	β-Fructofuranosidase		245, 246
	Glucose isomerase Glucose oxidase Lysozyme Penicillin amidase	Active immobilized enzyme	246

	Streptomyces phaeochromogenes cells	Active immobilized form of glucose isomerase	247
	Urease	Active immobilized enzyme	246
Concanavalin A	Agarose cyclic imidocarbonate	Investigation of mitogenic activity of an agarose–concanavalin A complex on rabbit spleen cells	92
		Isolation of glycogen (starch) synthetases by affinity chromatography	51
		Purification of human α_1-antitrypsin by affinity chromatography	52, 55
		Purification of human α-fetoprotein by affinity chromatography	53
		Purification of phytohaemagglutinins from lima bean (*Phaseolus lunatus*), soy bean (*Glycine max*), wax bean (*P. vulgaris*), and *Bandeisaea simplicifolia* by affinity chromatography	54
		Separation of monosaccharides and their derivatives and oligosaccharides; fractionation of mixtures of polysaccharides	56, 57
Fetuin	Agarose cyclic imidocarbonate	Production of immobilized gangliosides	61
Human fibrinogen glycopeptide	Agarose cyclic imidocarbonate	Demonstration of preparation of affinity chromatography materials using glycopeptides	77
Human haptoglobin	Agarose cyclic imidocarbonate	Removal of human haemoglobin from haemolysed sera	67
Human immunoglobulin IgM	Cellulose *trans*-2,3-cyclic carbonate	Isolation of anti-human immunoglobulin IgM by immunoadsorption	40
Normal human serum proteins	Agarose cyclic imidocarbonate	Removal of antibodies to light-chains from anti-human immunoglobulin IgM sera	39
		Removal of antibodies to normal serum proteins from anti-hepatitis sera	39

Table 6 (*cont.*)

Glycoprotein	*Macromolecule of matrix coupled*	*Use of product*	*Ref.*
Human pituitary follicle-stimulating hormone	Cellulose *trans*-2,3-cyclic carbonate	Measurement of human follicle-stimulating hormone by radioimmunoassay	146
Human serum albumin	Dextran cyclic carbonate	Investigation of the reaction of dextran cyclic carbonate with amino-compounds	210
Human serum albumin (^{125}I-labelled)	Cellulose *trans*-2,3-cyclic carbonate	Investigation of the reaction of cellulose *trans*-2,3-cyclic carbonate with amino-compounds	143
Immunoglobulin IgG	Agarose cyclic imidocarbonate	Removal of antibodies to light-chains of immunoglobulins from an antiserum containing antibodies to both μ- and light-chains	39
Lens culinaris phytoheamagglutinin	Agarose cyclic imidocarbonate	Purification of virus glycoproteins by affinity chromatography	72
Rabbit immunoglobulin IgG	Agarose cyclic imidocarbonate Cellulose *trans*-2,3-cyclic carbonate	Isolation of anti-rabbit immunoglobulin IgG by immunoadsorption	40
Thyroglobulin	Agarose cyclic imidocarbonate	Production of immobilized gangliosides	61
Wheat-germ agglutinin	Poly[*N*-4-diazobenzamidoethyl-(acrylamide)]	Investigation of the activity and specificity of immobilized agglutinins towards cell surfaces	258

slides that had been activated by a magnetic field, followed by coating with a membrane.[257] Homologous antibodies were still able to form a complex with the immobilized antigens (*e.g.* bovine serum albumin). It is assumed that the antigen–antibody combination is a sandwich-like complex having the antigen and antibody separated by the membrane. The fact that a long-range order of the nickel surface promoted a long-range interaction indicates that co-operative interactions among the antigens must occur, and that the reaction should be considered as an assembly of molecules reacting as a whole. It is assumed that the interactions involve specific Lifshitz–van der Waals forces.

An immobilized and denatured form of albumin has been prepared by coupling it to agarose cyclic imidocarbonate in the presence of urea; the product was examined as a material for the immobilization of gangliosides.[61]

Modification of Enzymes and Uses of Modified Enzymes

An attempt has been made to predict theoretically and quantitatively the change in action pattern that accompanies modification of one of the subsite affinities of a polymer-degrading system.[259] The hydrolyses of linear malto-oligosaccharides by α-amylase (from Taka amylase) were chosen as model systems since all the subsite affinities of the enzyme system are known. Calculations showed that a variety of action patterns could result from chemical modification at one of the subsites.

A thermolabile α-galactosidase from human placenta has been desialylated by treatment with neuraminidase.[260] Modification in this way did not change the immunological or kinetic properties of the enzyme, so that the experimental results do not support the concepts that the thermolabile α-galactosidase is a neuraminosyl derivative of the thermostable form found in human placenta and that the two enzymes are related structurally.

Modification of a neuraminidase from *Clostridium welchii* at the tryptophanyl residues with either *N*-bromosuccinimide or 2-hydroxy-5-nitrobenzyl bromide resulted in loss of activity.[261] In view of this, it was suggested that the tryptophanyl residues maintain the intact structure of the protein necessary for enzymic activity.

The reactions of two masked thiol groups of porcine pancreatic α-amylase with 5,5′-dithiobis-(2-nitrobenzoate) followed first-order kinetics in the presence of H_4edta.[262] Since the reaction was not accelerated on prolonged incubation of the enzyme with H_4edta, local fluctuations in the polypeptide chain are presumed to control the rate-limiting step of the reaction. Either one intramolecular or two mixed disulphides were formed as a result of the reaction, depending on the relative concentrations of

[259] K. Hiromi, M. Ohnishi, and S. Shibata, *J. Biochem. (Japan)*, 1973, **74**, 397.
[260] E. Beutler and W. Kuhl, *J. Biol. Chem.*, 1972, **247**, 7195.
[261] H. Bachmayer, *F.E.B.S. Letters*, 1972, **23**, 217.
[262] M. Telegdi and F. B. Straub, *Biochim. Biophys. Acta*, 1973, **321**, 210.

reactants. Neither oxidation of the thiol groups nor formation of mixed (enzyme-2-nitro-5-mercaptobenzoate)disulphide influenced the activity of the enzyme, although its stability was decreased.

Glycopeptides obtained from reduced and carboxymethylated Taka α-amylase (by digestion with trypsin and chymotrypsin) have been used for sequence studies.[263] A reduced and carboxymethylated derivative of α-amylase from *Bacillus stearothermophilus* has also been prepared and used for molecular-weight studies.[264] α-Amylase from *B. subtilis* has been acylated with 4-nitrophenyl acetate; it was found that derivatization increased the thermal stability of the enzyme above 70 °C, but decreased it at temperatures below 67 °C.[265] A compensation effect was also observed on inactivation of the acylated amylases by heat (temperature of compensation 68 °C), and this effect was attributed to a change in conformation of the protein chain caused by acylation.

The activities of a 4-phenylazobenzoyl derivative of α-amylase (from Taka amylase) towards various substrates have been compared with those of the underivatized form.[266] With phenyl α-maltotrioside as substrate, the derivatized enzyme produced more phenol and less phenyl α-D-glucopyranoside than the native enzyme, but with maltopentaitol, the activities of the two forms were similar. From the data obtained, it was concluded that the 4-phenylazobenzoyl group had been introduced into the enzyme very close to the catalytic site, and that interaction of the phenyl group with the substrate produced differences in activity between the natural and modified forms of the enzyme. Further studies of this derivative indicated that the 4-phenylazobenzoyl groups of the modified enzyme are liberated slowly on storage at 0—4 °C at pH 6, causing the action pattern to revert to normal.[267]

Carboxymethylated amylo-1,6-glucosidase-1,4-α-glucan-4-α-glycosyltransferase has been prepared.[268]

From an investigation of the reaction of glycine methyl ester with glucoamylase (from *Aspergillus niger*) in the presence of a water-soluble carbodi-imide, it was concluded that two or three carboxylic acid groups are present at the active site of the enzyme and that modification thereof causes loss of activity.[269] Glucoamylase from *Rhizopus niveus* has been coupled to insulin using glutaraldehyde as the linking agent; the product was used in a fluorometric immunoassay of insulin.[214]

Succinylation of the free thiol and amino-groups of bovine testicular hyaluronidase has been reported.[270]

[263] S. Isemura and T. Ikenaka, *J. Biochem.* (*Japan*), 1973, **74**, 1.
[264] K. Yutani, *J. Biochem.* (*Japan*), 1973, **74**, 581.
[265] I. Urabe, H. Nanjo, and H. Okada, *Biochim. Biophys. Acta*, 1973, **302**, 73.
[266] K. Omichi, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **72**, 665.
[267] K. Omichi, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1973, **73**, 491.
[268] E. Y. C. Lee and J. H. Carter, *F.E.B.S. Letters*, 1973, **32**, 78.
[269] C. J. Gray and M. E. Jolley, *F.E.B.S. Letters*, 1973, **29**, 197.
[270] A. Y. Khorlin, I. V. Vikha, and A. N. Milishnikov, *F.E.B.S. Letters*, 1973, **31**, 107.

Modification of hen egg-white lysozyme by γ-irradiation under anhydrous conditions has been examined.[271] A number of physicochemical and enzymic properties of the modified enzyme were investigated and compared with those of both the natural enzyme and a complex of lysozyme and glycol-chitin. Aggregates arising from irradiation are considered to involve the active site of the enzyme, and it appears that damaged enzyme molecules suffer further modifications during fractionation, resulting in inactivation.

Two of the three tyrosyl residues and four of the seven amino-groups in hen egg-white lysozyme were shown to react with *N*-acetylimidazole.[272] Changes in the c.d. spectrum accompanying derivatization could be partially reversed by deacetylation of *O*-acetylated tyrosyl residues. The deacetylated lysozyme showed a pH-dependence of the ellipticity at 305 nm similar to that found for the natural enzyme, but with an upwards pH shift of 0.5 units. It is suggested that the state of the tryptophanyl residues is changed by acetylation of the tyrosyl residues and amino-groups.

Hen egg-white lysozyme has been labelled to various degrees by reaction with fluorescein isothiocyanate, and the enzymic activities, c.d. spectra, and fluorescence characteristics of the conjugates were studied.[273] The fluorescence spectrum varied considerably with the degree of labelling, and it was suggested that, at high degrees of labelling, there might be some interaction between fluorescein groups and aromatic amino-acid residues.

Modification of the amino-groups of hen egg-white lysozyme by guanidination, succinylation, or maleylation resulted in changes in the c.d. spectrum in the aromatic region and in the activity of the enzyme; it was suggested that the state of the tryptophanyl residues is also changed by these modifications.[274] The enzyme, which had been reduced at its disulphide bonds with 2-mercaptoethanol, reacted with iodoacetic acid to give the carboxymethylated derivative and with methyl 4-nitrobenzenesulphonate to give the methylated derivative; in the latter case, derivatization occurred at the cysteine residues.[275] O.r.d. and c.d. spectroscopy in water showed that the two derivatives are significantly unfolded relative to the natural enzyme. The stabilities and immunological properties of the modified forms of the enzyme were investigated.

The carboxy-groups of hen egg-white lysozyme have been coupled with sulphanilic acid (a chromophoric nucleophile) using 1-ethyl-3-dimethylaminopropylcarbodi-imide at pH 5; other carbodi-imides were less effective in the coupling reaction.[276] The reactivities of the carboxy-groups with the reagent were investigated under various conditions, and informa-

[271] D. J. Marciani and B. M. Tolbert, *Biochim. Biophys. Acta*, 1973, **302**, 376.
[272] Y. Nakae, K. Ikeda, T. Azuma, and K. Hamaguchi, *J. Biochem.* (*Japan*), 1972, **72**, 1155.
[273] M. Hiramatsu, N. Okabe, and K. Tomita, *J. Biochem.* (*Japan*), 1973, **73**, 971.
[274] Y. Nakae, K. Ikeda, and K. Hamaguchi, *J. Biochem.* (*Japan*), 1973, **73**, 1249.
[275] C. L. Lee and M. Z. Atassi, *Biochemistry*, 1973, **12**, 2690.
[276] K. J. Kramer and J. A. Rupley, *Arch. Biochem. Biophys.*, 1973, **156**, 414.

tion was obtained on the involvement of the various carboxy-groups at the active site of the enzyme.

In a study of the reaction of turkey egg-white lysozyme with tetranitromethane at pH 8.5, it was found that the number of 3-nitrotyrosyl residues formed depends on the molar excess of the reagent, and that, at the highest excess, one tyrosyl residue remains unchanged.[277] At high reagent : enzyme ratios, tryptophanyl residues were also nitrated. Gel filtration indicated that polymerization of lysozyme accompanied nitration.

Holo- and apo-variants of glucose oxidase from *Aspergillus niger* have been modified at the tryptophanyl residues by treatment with 2-hydroxy-5-nitrobenzyl bromide and at the tyrosyl residues by treatment with *N*-acetylimidazole.[278] The effects of these modifications on the activity and fluorescence of the enzyme were investigated.

A labelled derivative of aminopeptidase (cytosol) has been prepared by reaction with fluorescein isothiocyanate, and the derivative was used to locate the enzyme following immobilization with water-insoluble, cyclic imidocarbonates of polysaccharides.[31]

Modification of the lysyl and tyrosyl residues of pineapple-stem bromelain by maleylation and by acetylation, respectively, decreased the amount of enzyme precipitated with anti-bromelain (stem), although the enzymic activity of the precipitate was largely preserved.[279] On the other hand, alkylation of cysteinyl residues at the active site resulted in almost complete loss of enzymic activity, although the antigenicity was unimpaired. It was concluded that the reaction of the enzyme with its homologous antibody does not involve the active site.

Human ceruloplasmin has been tritiated by reductive methylation of the ε-amino-group of lysyl residues with formaldehyde and sodium borotritide.[250] The derivatized form retained its oxidase activity and it appeared that derivatization did not alter the molecular size. The modified ceruloplasmin exhibited a plasma half-life in rats similar to that obtained when the glycoprotein was labelled in other ways. Moreover, reductive methylation did not alter the property of the enzyme to disappear promptly from circulation after desialylation. In a study of the reaction of nitric oxide with oxidized human ceruloplasmin, it was shown that nitric oxide is capable of forming a diamagnetic charge-transfer complex with type-1 copper atoms, whereas type-2 copper atoms are unaffected.[280] From e.s.r. spectroscopy of the derivative, it was possible to assign types to each of the copper atoms in the ceruloplasmin molecule.

The effects of reaction with maleic anhydride on the function and conformation of bovine α-lactalbumin have been investigated.[281] All the

[277] W. L. Riggle, J. A. Long, and C. L. Borders, jun., *Canad. J. Biochem.*, 1973, **51**, 1433.
[278] H. Tsuge and H. Mitsuda, *J. Biochem.* (*Japan*), 1973, **73**, 199.
[279] M. Sasaki, S. Iida, and T. Murachi, *J. Biochem.* (*Japan*), 1973, **73**, 367.
[280] R. Wever, F. X. R. Van Leeuwen, and B. F. van Gelder, *Biochim. Biophys. Acta*, 1973, **302**, 236.
[281] B. Kitchen and T. E. Barman, *Biochim. Biophys. Acta*, 1973, **298**, 861.

amino-groups of the molecule reacted to furnish a product that was highly acidic and highly expanded compared with the parent macromolecule. Despite these differences of physical properties, the maleylated derivative was as effective as natural α-lactalbumin in the lactose synthetase reaction, whereas reaction of α-lactalbumin with trinitrobenzenesulphonic acid resulted in inactivation.

Acetylation or succinylation of isoenzymes of alkaline phosphatase from human placenta increased their electrophoretic mobilities to different extents.[282] The electrophoretic mobilities were also increased, albeit to the same extent, by nitration or by treatment with *N*-ethylmaleimide.

The modification of enzymes to yield active, water-insoluble derivatives has continued to receive attention. Published versions of papers presented at an Engineering Foundation Conference on Enzyme Engineering have reported the immobilization of enzymes and the uses thereof in reactors.[283] Kinetic aspects of bound and free forms of enzymes were compared. The immobilization of enzymes by covalent attachment to water-insoluble, functionalized polymers, by intermolecular cross-linking with multifunctional reagents, by adsorption on to water-insoluble materials, by entrapment within cross-linked polymers, by microencapsulation, and by containment within semipermeable membranes has been reviewed.[284] The preparation of enzyme reactors and the applications of immobilized enzymes were also summarized. Another review on immobilized enzymes covers the following aspects: the carriers used (cellulose, cellulose derivatives, other polysaccharides, and organic and inorganic polymers, *etc.*), the properties (stabilities and kinetic patterns) of bound enzymes, methods of application (stirred tank reactors and columns, *etc.*), and potential applications in industry and medicine, *etc.*[28] An article on reactor separators incorporating membrane-bound enzymes has described their potential uses by increasing the membrane flux and by the use of multienzyme systems and co-factor and affinity-site membranes.[285] Applications to analyses were also discussed. The use of immobilized enzymes for affinity chromatography, including a number of specific applications, has been reviewed.[30]

Attention has been drawn to the importance in biology and engineering of steady-state, non-linear, diffusion equations describing reactions in constrained enzyme solutions.[286] Although, as in other types of non-linear, differential equations, exact analytical solutions do not exist except in simplified cases, a general procedure has been presented for solving numerically for the substrate-concentration profile and for the effectiveness factor of immobilized-enzyme catalysts; design correlations for enzyme

[282] D. M. Thomas and D. W. Moss, *Enzymologia*, 1972, **41**, 66.
[283] 'Enzyme Engineering', ed. L. B. Wingard, Interscience, New York, 1972.
[284] O. R. Zaborsky, 'Immobilized Enzymes', CRC Press, Cleveland, 1973.
[285] S. A. Barker and R. F. Burns, *Chem. and Ind.*, 1973, 801.
[286] D. J. Fink, T.-Y. Na, and J. S. Schultz, *Biotechnol. Bioeng.*, 1973, **15**, 879.

solutions constrained within spherical membranes were included. The use of a unique definition of the Thiele modulus in the charts permitted the effects of the concentration of substrate and the external, mass-transfer resistances on the overall effectiveness factor of the catalyst particle to be illustrated. On the basis of theoretical treatments of the kinetics of solid-supported enzymes in which diffusion effects are significant, methods have been suggested for analysing the experimental results; the procedures can be applied to membranes, spherical particles, and rods.[287] The Michaelis–Menten law applies to such systems, but the kinetic parameter K_m is only an apparent one, since it is influenced by partitioning and diffusional effects. The methods suggested allow the true kinetic parameters, relating to the behaviour of the enzyme within the support, to be derived from the experimental results. One method is applicable when the experimental results relate to various concentrations of substrate at either constant membrane thickness or particle diameter, whereas other methods are useful when the reverse situation pertains. The influence of diffusion on the apparent thermal stability of reversibly or irreversibly denaturable enzymes has been examined theoretically for uniform distribution of the enzyme in a porous solid.[288] If the overall reaction rate is influenced by diffusion through the catalyst, the insolubilized catalyst was shown to yield a form of apparently higher thermal stability, even though the maximal velocity and K_m values are the same for the free and insolubilized forms. Brief attention was given to the experimental conditions necessary to demonstrate conclusively whether or not insolubilization affects the thermal stability of an enzyme.

Many preparations of immobilized enzymes and of enzymes derivatized by coupling to other macromolecules to give water-soluble and -insoluble forms have been reported during the past year. These derivatives, together with their applications, are summarized in Table 7.[289–311]

Further details of carbohydrases and carbohydrate oxidases, isomerases, and synthetases worthy of mention are given below. The kinetics of β-fructofuranosidase immobilized by reaction with pellets of the diazo-derivative of a polyarylamine resin have been investigated for a packed-bed reactor.[297] At low flow-rates, an effect of interparticle diffusion on the K_m value was observed, and a correlation was proposed to evaluate this effect. The K_m and inhibition constants were determined at high flow-rates and compared with those of the native enzyme. Interparticle diffusion was found to be important with large pellets, and, at high concentrations of substrate, the effectiveness factor exceeded unity. Good agreement was found between the theoretical and experimental data. It was found that a cross-linking agent, such as *NN'*-methylenebisacrylamide, is unnecessary when β-fructofuranosidase, β-galactosidase, and glucoamylase are immobilized by γ-irradiation with acrylamide monomer.[294] The product gels

[287] T. Kobayashi, *Biochim. Biophys. Acta*, 1973, **302**, 1.
[288] D. F. Ollis, *Biotechnol. Bioeng.*, 1972, **14**, 871.

Table 7 *Modification of enzymes by coupling to insoluble matrices and other macromolecules*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Alcohol dehydrogenase	1.1.1.1	Glutaraldehyde cross-linked with albumin to give porous particles		
		Glutaraldehyde cross-linked with inactive proteins (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
L-Amino-acid oxidase	1.4.3.2	Glutaraldehyde cross-linked with albumin to give porous particles		
		Glutaraldehyde cross-linked with inactive proteins (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
α-Amylase	3.2.1.1	Entrapment in polyacrylamide by photopolymerization in the presence of acrylamide, cross-linking monomer, allyl glycidyl ether, glycidyl acrylate, and an oxygen scavenger		289
		Glutaraldehyde cross-linked within a matrix of silk	Active insoluble enzyme	96
		Titanous and titanic chelates of poly(4- and 5-acrylamidosalicylic acids)		290
β-Amylase	3.2.1.2	Reaction with epichlorohydrin cross-linked agarose derivative [see (35) and (35B), Scheme 14]	Active insoluble enzyme	33 [c]
Arylsulphatase	3.1.6.1	Concanavalin A	Active insoluble enzyme	244
Asparaginase	3.5.1.1	Glutaraldehyde cross-linked with albumin to give porous particles		96
		Microencapsulation in semipermeable membranes of cellulose nitrate	Active insoluble enzyme	172
		Microencapsulation in nylon and polyurea		291

[289] H. M. Walton and J. E. Eastman, *Biotechnol. Bioeng.*, 1973, **15**, 951.
[290] J. F. Kennedy and J. Epton, *Carbohydrate Res.*, 1973, **27**, 11.
[291] T. Mori, T. Tosa, and I. Chibata, *Biochim. Biophys. Acta*, 1973, **321**, 653.

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Aspartate ammonia-lyase	4.3.1.1	Adsorption on to calcium phosphate and silica gels		
		Carboxymethylcellulose azide		
		4-Diazobenzylcellulose		
		Entrapment in polyacrylamide gel	Active insoluble enzyme	139
		Ionic binding to diethylaminoethyl-, triethylaminoethyl-, and epichlorohydrin triethanolamine-cellulose, and diethylaminoethyl-Sephadex		
Carbamyl phosphokinase	—	Glutaraldehyde cross-linked with alkylamine derivatives of glass	Continuous production of adenosine triphosphate from adenosine diphosphate, phosphate, and cyanate	292
Carbonate dehydratase	4.2.1.1	Glutaraldehyde cross-linked inside a matrix of silicone		
		Glutaraldehyde cross-linked with albumin to give porous particles	Active insoluble enzyme	96
Catalase	1.11.1.6	Glutaraldehyde cross-linked inside a matrix of activated carbon		96
		Microencapsulation in collodion membranes		173
		Glutaraldehyde cross-linked with albumin to give porous particles		96
		Glutaraldehyde cross-linked with inactive proteins (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
		Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly(vinyl chloride), and γ-methylpolyglutamate		133 [b]
Chymotrypsin	3.4.21.1	Agarose cyclic imidocarbonate		34, 34 [c]
		Azido- and diazo-derivatives of copolymers of ethylene and maleic anhydride	Active insoluble enzyme	293

		Carboxymethylcellulose azide	Thermal denaturation studies of the enzyme	153
		Cyclic imidocarbonate derivative of extruded cellulose containing iron oxide		196
		Glutaraldehyde cross-linked inside a matrix of cellophane membrane		96
		Macroporous cellulose *trans*-2,3-cyclic carbonate	Active insoluble enzyme	148
		Precipitated iron oxide activated with 3-aminopropyltriethoxysilane		196
Dextranase	3.2.1.11	Carboxymethylcellulose azide		
		Cellulose *trans*-2,3-cyclic carbonate	Active insoluble enzyme	145
		Cellulose *trans*-2,3-cyclic imidocarbonate		
β-Fructofuranosidase	3.2.1.26	Ionic adsorption to diethylaminoacetyl-cellulose		157
		Casting admixed with collagen on a nylon support then cross-linked with glutaraldehyde		245
		Entrapment within poly(acrylamide) by γ-irradiation in the presence of acrylamide		294
		Entrapment within poly(vinyl alcohol) matrices by electron beam irradiation		295
		Entrapment within poly(vinyl alcohol) matrices by γ-irradiation	Active insoluble enzyme	296
		Impregnation of collagen with the enzyme		246
		Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly-(vinyl chloride), and γ-methylpolyglutamate		133, 133 [b]
		Polydiazo derivative of ion-exchange resin		297

[393] D. L. Marshall, *Biotechnol. Bioeng.*, 1973, **15**, 447.
[394] L. Goldstein, *Biochim. Biophys. Acta*, 1973, **315**, 1.
[394] H. Maeda, A. Yamauchi, and H. Suzuki, *Biochim. Biophys. Acta*, 1973, **315**, 18.
[395] H. Maeda, H. Suzuki, and A. Yamauchi, *Biotechnol. Bioeng.*, 1973, **15**, 827.
[396] H. Maeda, H. Suzuki, and A. Yamauchi, *Biotechnol. Bioeng.*, 1973, **15**, 607.
[397] T. Kobayashi and M. Moo-Young, *Biotechnol. Bioeng.*, 1973, **15**, 47.

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Fructose biphosphate aldolase	4.1.2.13	Agarose cyclic imidocarbonate	Active insoluble enzyme	35
β-Galactosidase	3.2.1.23	Adsorption on catechol–formaldehyde, 2-cresol–phenol–formaldehyde, 4-hydroxybenzaldehyde diethylacetal–formaldehyde, oxidized 2-cresol–phenol–formaldehyde, periodate-oxidized catechol, phloroglucinol–formaldehyde, resorcinol–formaldehyde, and salicylaldehyde diethylacetal–formaldehyde polymers, charcoal, Duolite S-30 derivatives, humic acid, mixed primary and secondary amine ion-exchange resins, and phenol–formaldehyde resin	Active insoluble enzyme	298
		trans-2,3-Cyclic imidocarbonate derivative of extruded cellulose containing iron oxide		196
		Diazo-derivative of glass		299
		Entrapment within poly(acrylamide) by γ-irradiation in the presence of acrylamide		294
		Entrapment within poly(acrylamide) by bead polymerization		300
		Glutaraldehyde cross-linked within a matrix of cellophane	Inactive insoluble enzyme	96
		Glutaraldehyde cross-linked with inactive proteins (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
		Microencapsulation using 1,6-diaminohexane and terephthaloyl dichloride		301
		Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly(vinyl chloride), and γ-methylpolyglutamate		133
		Precipitation with iron oxide activated with 3-aminopropyltriethoxysilane		196

Glucoamylase	3.2.1.3	Bromoacetylcellulose	Active insoluble enzyme	142
		Cellulose *trans*-2,3-cyclic imidocarbonate		163
		Diazo-derivative of porous glass		302
		Entrapment in films of cellulose acetate		134
		Entrapment in polyacrylamide by photo-polymerization in the presence of acrylamide, cross-linking monomer, allyl glycidyl ether, glycidyl acrylate, and oxygen scavenger		289
		Entrapment within poly(acrylamide) by γ-irradiation in the presence of acrylamide		294
		Entrapment within poly(vinyl alcohol) matrices by electron-beam irradiation		295
		Entrapment within poly(vinyl alcohol) matrices by γ-irradiation		296
		Glutaraldehyde cross-linked to aminoalkyl derivatives of porous glass		302
		Iodoacetylcellulose		142
		Poly(diazostyrene)		303
		Titanous and titanic chelates of poly(4- and 5-acrylamidosalicylic acids)		290
Glucose isomerase	5.3.1.18	Alkaline formaldehyde and glutaraldehyde cross-linked to collagen	Active insoluble enzyme	247 [d]
		Electrodeposition of the enzyme with collagen		246
		Physical entrapment in filamentous structures		304

[298] W. L. Stanley and R. Palter, *Biotechnol. Bioeng.*, 1973, **15**, 597.
[299] J. H. Woychik and M. V. Wondolowski, *Biochim. Biophys. Acta*, 1972, **289**, 347.
[300] A. Dahlqvist, B. Mattiasson, and K. Mosbach, *Biotechnol. Bioeng.*, 1973, **15**, 395.
[301] J. C. W. Østergaard and S. C. Martiny, *Biotechnol. Bioeng.*, 1973, **15**, 561.
[302] D. R. Marsh, Y. Y. Lee, and S. T. Tsao, *Biotechnol. Bioeng.*, 1973, **15**, 483.
[303] W. M. Ledingham and M. do Socorro Santos Ferreira, *Carbohydrate Res.*, 1973, **30**, 196.
[304] S. Giovenco, F. Morisi, and P. Pansolli, *F.E.B.S. Letters*, 1973, **36**, 57.

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Glucose oxidase	1.1.3.4	Electrodeposition of the enzyme with collagen		246
		Glass silanized with γ-aminopropyltriethoxysilane, then allowed to react with thiophosgene		305
		Glutaraldehyde cross-linked within a matrix of cellophane		96
		Glutaraldehyde cross-linked with inactive proteins (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
		Physical entrapment in filamentous structures using cellulose acetate, ethylcellulose, poly(vinyl chloride), and γ-methylpolyglutamate		133 [b, e]
		Poly(vinyl 4-aminophenoxyacetate)	Active soluble enzyme	306
		Titanous chelates of alginic acid, chitin, and Celite	Active insoluble enzyme	102
Glucose phosphate isomerase	5.3.1.9	Glutaraldehyde cross-linked with inactive 'protein' (albumin, fibrinogen, and haemoglobin) to give membranes	Active insoluble enzyme	96
β-Glucosidase		Cellulose *trans*-2,3-cyclic carbonate		142
		Diethylaminoethylcellulose *trans*-2,3-cyclic carbonate	Active insoluble enzyme	159
Hexokinase	2.7.1.1	Glutaraldehyde cross-linked within a matrix of aminated paper		
		Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
α-Lactalbumin	see 2.4.1.22	Agarose cyclic imidocarbonate	Purification of *N*-acetyllactosamine synthetase by affinity chromatography	69

Lactate dehydrogenase	1.1.1.27	Diazoaryl derivative of glass		307
		Glutaraldehyde cross-linked to aminoalkyl derivatives of glass		307
		Glutaraldehyde cross-linked with albumin to give porous particles	Active insoluble enzyme	96
		Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes		96
Lysozyme	3.2.1.17	4-Diazobenzylcellulose	Active insoluble enzyme	140
		Imidazole derivation of copolymer of 4-vinylbenzoic acid and styrene	Demonstration of production of a matrix-supported enzyme in non-aqueous conditions	308
		Impregnation of collagen with the enzyme		246
		Poly[*N*-4-diazobenzoyl(*N*-2-aminoethylacrylamide) amidoethyl(acrylamide)]	Active insoluble enzyme	140
Papain	3.4.22.2	Azido- and diazo-derivatives of copolymers of ethylene and maleic anhydride	Active insoluble enzyme	293
		Acid chloride derivative of glass coated with zirconium oxide		
		Diazo-derivative of glass coated with zirconium oxide	Active insoluble enzyme	309
		Glutaraldehyde cross-linked to aminoalkyl derivatives of glass coated with zirconium oxide		
Penicillin acylase	—	Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly(vinyl chloride), and γ-methylpolyglutamate	Active insoluble enzyme	133

[305] B. J. Rovito and J. R. Kittrell, *Biotechnol. Bioeng.*, 1973, **15**, 143.
[306] H. F. Hixson, *Biotechnol. Bioeng.*, 1973, **15**, 1011.
[307] J. E. Dixon, F. E. Stolzenbach, J. A. Berenson, and N. O. Kaplan, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 905.
[308] G. J. Bartlin, H. D. Brown, and S. K. Chattopadhyay, *Nature*, 1973, **243**, 342.
[309] H. H. Weetall and R. D. Mason, *Biotechnol. Bioeng.*, 1973, **15**, 455.

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Penicillin amidase	3.5.1.11	2-Amino-4-chloro-*s*-triazinyl derivative of diethylaminoethylcellulose	Active insoluble enzyme	160
		Impregnation of collagen with the enzyme		246
Peroxidase	1.11.1.7	Glutaraldehyde cross-linked within a matrix of cellulose		96
		Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
		Physical entrapment in filamentous structures using cellulose acetate, ethylcellulose, poly(vinyl chloride), and γ-methylpolyglutamate		133 [e]
Phenylalanine decarboxylase	4.1.1.53	Glutaraldehyde cross-linked with albumin to give porous particles	Active insoluble enzyme	96
Phosphorylase	2.4.1.1	Adsorbed on to colloidal silica and poly(ethyleneimine)-coated silica		
		2-Amino-4-chloro-*s*-triazinyl derivative of porous glass	Active insoluble enzyme and polysaccharide synthesis	5
		Glutaraldehyde cross-linked to aminoalkyl derivatives of porous glass		
Polyribonucleotide	2.7.7.8	Agarose cyclic imidocarbonate		
		Cellulose *trans*-2,3-cyclic imidocarbonate	Active insoluble enzyme	36
		Glutaraldehyde cross-linked to aminoalkyl derivatives of glass		
Polygalacturonase	3.2.1.15	Titanous and titanic chelates of poly(4- and 5-acrylamidosalicylic acids)	Inactive insoluble enzyme	290
Pronase	3.4.21.4/ 3.4.24.4	Carboxymethylcellulose azide	Active insoluble enzyme and prevention of autodigestion	160
		Cellulose *trans*-2,3-cyclic imidocarbonate		
		Ethylene–maleic anhydride copolymer		

Ribonuclease I	3.1.4.32	Agarose cyclic imidocarbonate	Active insoluble enzyme	37 [c]
Ribonuclease	3.1.4.22/23	Glutaraldehyde cross-linked with albumin to give porous particles		
		Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
Subtilisin	3.4.21.14	Azido- and diazo-derivatives of copolymers of ethylene and maleic anhydride		293
		Diazotized Sephadex anthranilate (see Scheme 20)	Active insoluble enzyme	213
Triose phosphate isomerase	5.3.1.1	Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes	Active insoluble enzyme	96
Trypsin	3.4.21.4	Arylazo-derivative of glass	Active insoluble enzyme and active-site titration method for immobilized enzyme	310
		Azido- and diazo-derivatives of copolymers of ethylene and maleic anhydride		293
		Cellulose *trans*-2,3-cyclic carbonate		144
		Diazotized Sephadex anthranilate (see Scheme 20)	Active insoluble enzyme	213
		Glutaraldehyde cross-linked within a matrix of cellophane		96
Tryptophan synthetase	4.2.1.20	Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly-(vinyl chloride), and γ-methylpolyglutamate	Active insoluble enzyme	133
Tyrosine phenol-lyase	4.1.99.2	Physical entrapment in filamentous structures of cellulose acetate, ethylcellulose, poly-(vinyl chloride), and γ-methylpolyglutamate	Active insoluble enzyme	133

[310] J. R. Ford, R. P. Chambers, and W. Cohen, *Biochim. Biophys. Acta*, 1973, **309**, 175.

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Urate oxidase	1.7.3.3	Glutaraldehyde cross-linked within a matrix of cellophane	Active insoluble enzyme	96
		Glutaraldehyde cross-linked with albumin to give porous particles		
		Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, or haemoglobin) to give membranes		
Urease	3.5.1.5	Glutaraldehyde cross-linked and then copolymerized with acrylamide, bisacrylamide, *N'N'N''N''*-tetramethylethylenediamine, and agarose		96
		Glutaraldehyde cross-linked with albumin to give porous particles	Active insoluble enzyme	96
		Impregnation of collagen with the enzyme		246
		Microencapsulation in nylon		311
		Microencapsulation in semipermeable collodion membranes		173
Xanthine oxidase	1.2.3.2	Glutaraldehyde cross-linked with inactive protein (albumin, fibrinogen, and haemoglobin) to give membranes	Active insoluble enzyme	96

[a] Generally a covalent coupling may be assumed unless otherwise stated; [b] Mixed enzyme derivative of catalase, β-fructofuranosidase, and glucose oxidase; [c] Agarose beads cross-linked with epichlorohydrin; [d] Using whole cells of *Streptomyces phaeochromogenes*; [e] Mixed enzyme derivative of glucose oxidase and peroxidase.

[211] P. V. Sundaram, *Biochim. Biophys. Acta*, 1973, **321**, 319.

were rigid, possessed satisfactory activities, and no leakage of the enzyme was detectable. Analogous entrapment of β-fructofuranosidase and glucoamylase by γ-irradiation with vinyl alcohol also gave stable, immobilized forms of the enzymes, which are considered more likely to find applications in medicine in view of the greater compatibility of poly(vinyl alcohol) than polyacrylamide with living organisms.[296] More active immobilized forms of β-fructofuranosidase and glucoamylase were obtained when entrapment within poly(vinyl alcohol) was achieved by irradiation with an electron beam.[295] These derivatives of the enzymes were as stable as the previous derivatives, and it was assumed that the accelerated electron beam promoted a greater extent of cross-linking. Immobilized forms of β-fructofuranosidase and lysozyme have been prepared by impregnation of a pre-swollen membrane of collagen with a solution of the enzyme, and glucose oxidase and glucose isomerase have been immobilized by electrodeposition from a dispersion of collagen containing the dissolved enzyme.[246] The membranous complexes were used to construct biocatalytic reactors, which were employed in a recirculation system for the conversion of appropriate substrates. The reactors showed initial decreases of activity to stable limits, which were then maintained over a large number of replacements of the reactor volume. The mechanism of complex formation between collagen and the enzymes involves multiple ionic linkages, hydrogen bonds, and van der Waals interactions. The immobilized enzyme obtained by ionic binding of β-fructofuranosidase to diethylaminoacetylcellulose was stable to electrolytes in the pH range 5—7 and up to 45% of the enzymic activity was retained on binding.[157] The stability to heat and the effect of temperature on the activity of the complex were almost identical with those of the native enzyme.

Novel types of immobilized glycosidases, *etc.* have been provided in fibre-entrapped forms.[133] Addition of an aqueous solution of an enzyme to a solution of a polymer [cellulose acetate, ethylcellulose, poly(vinyl chloride), or γ-methylpolyglutamate] in a solvent immiscible with water produced an emulsion that could be extruded through a spinnaret into a coagulation bath. In this wet-spinning process, the enzyme remains entrapped within the pores of the fibre, the size of which can be controlled. Immobilized derivatives of β-fructofuranosidase and β-galactosidase, and mixed derivatives of β-fructofuranosidase, glucose oxidase, and catalase, and of glucose oxidase and peroxidase have been produced in this way; it was also demonstrated that the fibres can be woven into fabrics.

Coupling of a β-galactosidase to glass by diazo-linkages caused only a 25% loss of enzymic activity, and the properties of the enzyme did not appear to be affected by immobilization.[299] β-Galactosidase that had been immobilized by cross-linking with polyacrylamide using a bead-polymerization technique has been used for the preparation of lactose-free milk.[300] Methods for immobilization of β-galactosidase on a number of

matrices (including various phenolic resins) by one-step processes have been investigated.[298] β-Galactosidase immobilized in water-insoluble microcapsules (formed from 1,6-diaminohexane and terephthaloyl dichloride) has been shown to have significant stability in sera.[301] β-Galactosidase, α-amylase, and glucose oxidase immobilized by cross-linking with glutaraldehyde within either cellophane or silk sheets gave activity yields of up to 10%.[96] However, activity yields of up to 80% were obtained when the enzymes were immobilized in membrane form by cross-linking to albumin, fibrinogen, or haemoglobin with glutaraldehyde, or by cross-linking to albumin with glutaraldehyde to give porous particles. Immobilized derivatives of β-galactosidase have also been obtained by treating the enzyme with iron oxide activated with 3-aminopropyltriethoxysilane (see also p. 547).[196]

Preparations of β-glucosidase attached to cellulose *trans*-2,3-cyclic carbonate with improved activity have been described.[144] The maximum amount of enzymic activity was coupled in the pH range 7—8, but the optimum pH for coupling to DEAE-cellulose *trans*-2,3-cyclic carbonate was 9—10 (see p. 538).[159]

The coupling of α-amylase, glucoamylase, and polygalacturonase to titanous and titanic chelates of water-insoluble poly(*N*-acryloyl-4- and -5-aminosalicylic acids) is envisaged to occur by replacement of water ligands surrounding the titanium atoms by amino-groups of the protein chain of the enzyme.[290] The products obtained from α-amylase and glucoamylase are enzymically active; whereas α-amylase was easily washed off, the glucoamylase derivative withstood extensive washing and could be used continuously in a column. A particular advantage of the glucoamylase derivative is that the coupling can be achieved within 1 hour to give a product that retains a high specific activity against macromolecular substrates. The inactivity of the polygalacturonase derivative was attributed to inhibition of the enzyme by the matrix which, in a sense, resembles a polygalacturonic acid. The partial hydrolysis of starch has been accomplished using α-amylase and glucoamylase that had been immobilized by photopolymerization in aqueous solutions containing acrylamide, a cross-linking monomer (allyl glycidyl ether), an enzyme-linking monomer (glycidyl acrylate), and an oxygen-scavenger.[289] Columns containing both entrapped enzymes were capable of producing 90—94% dextrose syrups.

Covalent fixation of β-amylase to amino-derivatives of macroporous agarose cross-linked with epichlorohydrin has been mediated by cyclohexyl isocyanide and acetaldehyde (see 35 and 35B, p. 516).[33] Retention of activity on immobilization was as high as 35%, and the activity–pH and –ionic strength profiles were essentially the same as those of the free enzyme, although the immobilized form was less stable to storage and to use. The enzyme derivative was used for the continuous degradation of starch.

The pH optima and action patterns of dextranase immobilized by attachment to cellulose *trans*-2,3-cyclic carbonate, cellulose cyclic imido-

carbonate, and carboxymethylcellulose azide were shown to be similar to those of the free enzyme, but increased stabilities were noted for the immobilized forms.[145]

Various properties, including its behaviour in packed-bed and stirred reactors, of glucoamylase immobilized by attachment to beads of diazotized poly(aminostyrene) have been described.[303] The effects of the type of cellulose used, the particle size, and the surface structure on the attachment of glucoamylase to cellulose cyclic imidocarbonate have been investigated; pre-grinding to give deep cuts on the surfaces of the cellulose particles increased the specific activity and protein content of the immobilized enzyme.[103] Pretreatment of halogenoacetylcelluloses with organic solvents permitted the binding of glucoamylase with up to 82% retention of the activity (*cf.* the free enzyme) against maltose; the highest activities were achieved by using the smallest particles.[131] A study has been made of the controlling mass-transfer resistance in the overall reaction rate for the conversion of maltose into D-glucose by glucoamylase immobilized by attachment to aminoalkyl- and diazo-derivatives of glass.[302] For normal operation of a packed column and an air-stirred batch reactor, the rate-controlling step was found to be the internal resistance to simultaneous pore diffusion and chemical reaction. Experimental effectiveness factors were determined and compared with those derived from a theoretical diffusion model based on Michaelis–Menten kinetics. Whereas the pH optima (pH 5) of the natural and azo-coupled enzymes were identical, the glutaraldehyde-linked enzyme possessed a lower pH optimum (pH 4.3). Glucoamylase has been entrapped in cellulosic fibres by wet-spinning of an emulsion obtained by mixing an aqueous solution of the enzyme with one of cellulose acetate in methylene chloride.[134] The effects of substrate concentration, temperature, and protein content on the activity of the immobilized enzyme were studied, and possible application of this glucoamylase to the conversion of starch into D-glucose was investigated.

The coupling of lysozyme to 4-diazobenzylcellulose has been carried out in the presence of 2-acetamido-2-deoxy-D-glucose, after which the residual diazo-groups on the matrix were quenched with 2-naphthol.[140] Coupling of the enzyme in this way was found to be partially reversible, whereas coupling to poly[*N*-4-diazobenzamidoethyl(acrylamide)] gave a stable attachment. The specific activity of immobilized lysozyme decreased with increasing density of derivatized groups on the surface, and this was explained in terms of deactivation of the protein. Other experiments on the immobilization of lysozyme have been designed to overcome the restricted range of chemical reactions available for immobilization in aqueous systems.[308] Because enzymes generally have a low solubility in organic solvents, it was convenient to perform the coupling reaction using a support that could be dissolved first in an appropriate organic solvent and then activated to yield a soluble matrix containing reactive groups, thereby binding the insoluble enzyme in a two-phase system. A linear copolymer

prepared from 4-vinylbenzoic acid and styrene displayed solubility characteristics consistent with the ultimate isolation of the copolymer–lysozyme complex by precipitation with a non-solvent.

In the use of titanium chelates of alginic acid, chitin, and Celite for the production of water-insoluble derivatives of glucose oxidase, covalent binding is considered to take place by the replacement of water ligands of the bound titanium atoms by ε-amino-groups of lysyl residues of the enzyme.[102] It was found that the immobilized enzyme could be stabilized to storage in the dry state by freeze-drying in the presence of D-glucitol, which appears to provide a hydrophilic environment for the bound enzyme. Carbodi-imide-mediated coupling of glucose oxidase to poly(vinyl 4-aminophenoxyacetate) has yielded a water-soluble immobilized form of the enzyme that could be purified by gel filtration.[306] The immobilized enzyme possessed greater thermal stability than the free enzyme. Quantitative studies have been reported on external-film and internal-pore diffusion effects for glucose oxidase immobilized on a derivatized porous glass.[305] Using a continuous, tubular, packed-bed reactor, it appeared that immobilized enzymes free from internal diffusional restrictions generally require the use of supports having pore-sizes larger than those currently available in porous glass.

A novel form of glucose isomerase has been prepared by immobilization of heat-treated cells of *Streptomyces phaeochromogenes* containing the enzyme.[247] The cells were bound physically to collagen and this material was then tanned with either alkaline formaldehyde or glutaraldehyde to the desired mechanical strength. The membranes obtained were in forms convenient for carrying out catalytic reactions, since penetration of the membrane into cell locales was facilitated by its highly swollen, open structure. Retention of a high level of activity by the immobilized derivative was demonstrated by continuous operation of a column packed with a tanned membrane of collagen and whole cells for 40 days at 70 °C. Glucose isomerase has also been immobilized by entrapment in filamentous structures (see p. 532), and the conditions for optimal activity and stability of the derivative were determined.[304]

Immobilized forms of phosphorylase have been prepared by adsorption on to either colloidal silica or poly(ethyleneimine)-coated silica, by reaction with a 2-amino-4-chloro-*s*-triazinyl derivative of porous glass, and by cross-linking with glutaraldehyde to an aminoalkyl derivative of porous glass.[5] Whereas the enzyme loading on the silica derivatives was greater, the porous-glass derivatives possessed superior physical properties for flow-through operations. Each of the immobilized enzyme preparations was capable of synthesizing polysaccharides over a wide range of molecular weights (up to the limit of solubility of the polysaccharide produced); the porous-glass derivatives were used for this purpose in a continuous operation (half life 28 days). The polysaccharide produced was found to accumulate around the enzyme–glass particles, but could be removed by the action of glucoamylase.

Modification of Gangliosides and Glycolipids, and Uses of Modified Gangliosides and Glycolipids

N-Hydroxysuccinimide esters and mixed anhydrides of various gangliosides have been prepared.[61] Immobilized forms of gangliosides were produced by reaction of these derivatives with poly(L-lysine), a branched, multi-chain copolymer of poly(L-lysine) and DL-alanine, albumin, denatured albumin, fetuin, thyroglobulin, and 3,3′-diaminodipropylamine, which had been allowed to react with the cyclic imidocarbonate derivative of macroporous agarose. The gangliosides were also coupled *via* their carboxy-groups to the agarose derivatives with the aid of a water-soluble carbodiimide or dicyclohexylcarbodi-imide. All the immobilized forms of the mixed gangliosides were tested for the affinity chromatography of *Vibrio cholerae* enterotoxin, and those possessing poly(amino-acid) or albumin bridges were most effective.

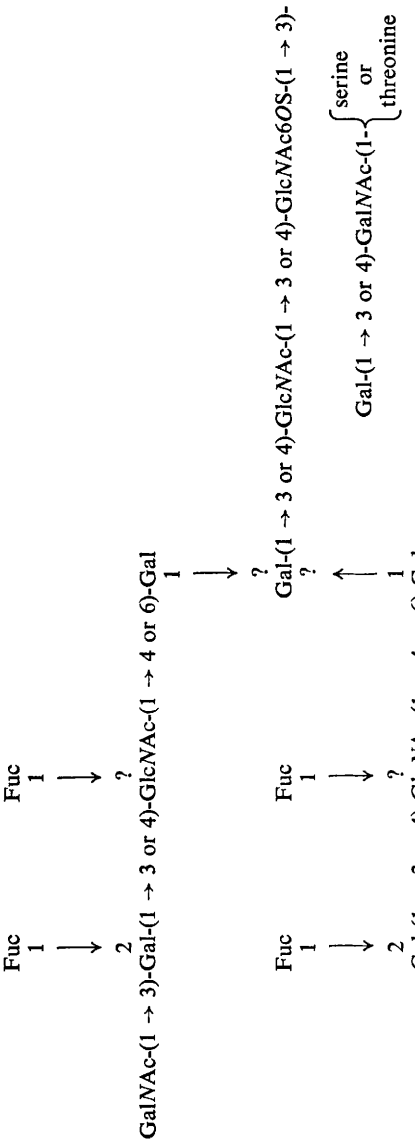
O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide has been obtained by enzymic treatment of the corresponding ganglioside.[312] The labelled ganglioside was prepared biosynthetically from *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide and UDP-2-acetamido-2-deoxy-D-[1-^{14}C]galactose with the aid of a UDP-acetamido-deoxygalactosyltransferase.

A D-galactosyldihexosylceramide has been labelled specifically at position 6 of the terminal D-galactosyl residues by oxidation with galactose oxidase, followed by reduction of the 6-aldehydo derivative with sodium borotritide.[313]

[312] D. A. Wenger, S. Okada, and J. S. O'Brien, *Arch. Biochem. Biophys.*, 1972, **153**, 116.
[313] M. W. Ho, *Biochem. J.*, 1973, **133**, 1.

Erratum

Vol. 6, 1973: *p.* 293. The structure below should replace that given for the A + H sulphated glycoprotein of hog gastric mucosa:



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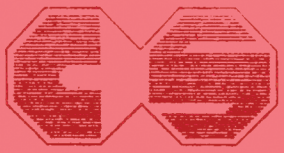
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