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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 2

A Review of the Literature Published during 1968

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Preface

Our aim in preparing the second volume in this series has been to cover the literature available between mid-January 1968 and mid-January 1969. The terms of reference are set out as before in the Introductions to Parts I and II. Our overall emphasis is the same as that described in the preface to Volume 1.

Work described in *Abstracts of the American Chemical Society Meetings*, in *Dissertation Abstracts*, or in the patent literature has not been abstracted.

In the preface for Volume 1 we requested comments and advice from readers. However, because of the delayed distribution of that volume we have had to prepare this manuscript having received virtually no comments at all.

It has been useful sometimes to give a reference to work described in Volume 1 and this is given, for example, in the form (Vol. 1, p. 247).

We thank Drs. O. S. Chizhov and L. V. Bakinovskii for help with the translation of Russian papers.

R. D. G.
R. J. F.
M. J. H.
P. J. S.

March 1969.

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Part I Mono-, Di-, and Tri-saccharides and their Derivatives

Part II Macromolecules

Abbreviations

The following abbreviations have been used

ATP	adenosine triphosphate
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DCC	dicyclohexylcarbodi-imide
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
e.s.r.	electron-spin resonance
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphoramide
i.r.	infrared
LAH	lithium aluminium hydride
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
p.l.c.	preparative thin-layer chromatography
py	pyridine
THF	tetrahydrofuran
t.l.c.	thin-layer chromatography
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

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

By

R. J. Ferrier

R. D. Guthrie

1
Introduction

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

There have been several significant developments during the year in glycoside chemistry: acetals have been detected in the methanolysis of sugars; methods for the synthesis of α-glucosides and -glucosaminides have been developed by Lemieux, as well as work on many other glycosidation methods by other groups. The Hanessian–Hullar reaction in which a 4,6-*O*-benzylidene acetal is converted into a 4-*O*-benzoyl-6-bromo-6-deoxy-compound has found wide use in synthetic sequences. New routes have been developed for the synthesis of D-gulose and D-lyxose, and the hitherto unknown, all-*cis*, methyl β-D-lyxofuranoside has been described. Many novel, naturally occurring derivatives have been reported, including the first natural nitro-sugar. Much study has been made of the anomeric effect and it is now becoming apparent that cyclohexane and tetrahydropyran systems may differ considerably with regard to conformational stabilities of their substituents. *x*-Ray diffraction techniques have been used more than ever before in carbohydrate chemistry and the number of papers using mass spectrometry as an analytical tool has increased.

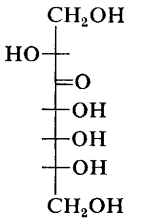
Apart from the first Volume of this Series, other books of interest to carbohydrate chemists were published during 1968. Volume 1F in the series 'Rodd's Chemistry of Carbon Compounds' provided an excellent up-to-date account of all aspects of carbohydrate chemistry.[1] A new edition [2] of an established undergraduate textbook appeared, and also a French translation [3] of it. A general text in Russian was also published.[4] To commemorate the retirement of Sir Edmund Hirst, a special number of *Carbohydrate Research* was issued.[5]

[1] 'Rodd's Chemistry of Carbon Compounds,' vol. 1F, Elsevier, 1968, 780 pp.
[2] R. D. Guthrie and J. Honeyman, 'Introduction to the Chemistry of Carbohydrates,' Oxford University Press, 3rd edn, 1968, 144 pp.
[3] R. D. Guthrie and J. Honeyman, 'Introduction à la Chimie des Glucides' (translated by G. Chapas), Dunod, Paris, 1968, 170 pp.
[4] N. K. Kochetkov, A. F. Bochkov, B. A. Dmitriev, A. J. Usov, O. S. Chizhov, and W. N. Shibaev, 'Khimia Uhlewodov' (Chemistry of the Carbohydrates), ed. M. N. Pastushenko and L. A. Panteleeva, Khimia, Moscow, 1967, 672 pp.
[5] *Carbohydrate Res.*, May 1968.

2
Free Sugars

Isolation and Synthesis

A new naturally occurring heptulose, named coriose, isolated from the leaves and stem of *Coriana japonica* A. Gray has been shown to be D-*altro*-3-heptulose (1) by chemical degradation [6] and by synthesis *via* the aldol



(1)

condensation of 2,4-*O*-ethylidene-D-erythrose.[7] The carbohydrates extractable from potato with 80% alcohol have been characterised as: D-galactose, D-glucose, D-fructose, *myo*-inositol, sucrose, melibiose, maltotriose, digalactosyl glycerol, glucosyl *myo*-inositol, raffinose, planteose, galactosyl *myo*-inositol, mannotriose, stachyose, and trigalactosyl glycerol. In addition small amounts of ribosyl, xylosyl, and arabinosyl glucose were detected, together with two trisaccharides, each containing fructose and glucose in the ratio 2 : 1.[8] Hydrolysis, chemical and enzymic, of sawdust and of cellulose has been examined as a possible means of obtaining the constituent monosaccharides for use in foodstuffs. From cellulose, D-glucose was obtained in only *ca.* 2·5% yield; sawdust gave D-glucose and D-xylose in yields of 4·1, and up to 6·7%, respectively.[9]

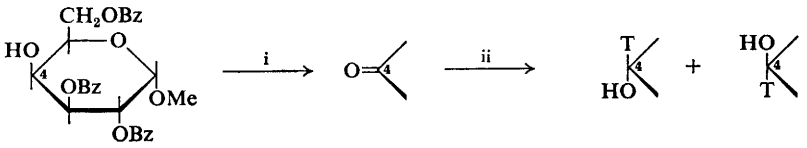
Methods for the synthesis of free sugars have again received attention. A new synthetic route to D-gulose has been devised (see p. 148), and an independent report has been made of a route to D-allulose (D-psicose) described last year (see p. 147); a synthesis of L-allulose has also been reported; 1,3-dideoxy-D-*erythro*-hexulose has also been synthesised (see p. 123). Two new routes to D-lyxose have been reported (see pp. 41

[6] T. Okuda and K. Konishi, *Chem. Comm.*, 1968, 553.
[7] T. Okuda and K. Konishi, *Tetrahedron*, 1968, **24**, 6907; *Chem. Comm.*, 1968, 671.
[8] B. Urbas, *Canad. J. Chem.*, 1968, **46**, 49.
[9] F. H. de Padilla and F. H. Hoskins, *J. Agric. Food Chem.*, 1968, **16**, 735.

and 73). Reduction of 7-*O*-acetyl-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-heptos-6-ulose (vol. 1, p. 139) with LAH and removal of the blocking groups gave L-*glycero*-D-*galacto*-heptose, identified by comparison with its known enantiomer.[10] A synthesis of L-fructose by the enzymic isomerisation of L-mannose (which is commercially available) has been reported.[11] Methods for the preparation of the commercially unavailable β-D-mannose and its penta-acetate have been described from the α-anomer and from ivory nut.[12] Methods for aldose chain extension have been reported (p. 136).

An industrial process for the synthesis of lactulose from lactose has been described. A combination of these two disaccharides is important in baby foods.[13]

Ready syntheses of D-[4-^{3}H]glucose and D-[4-^{3}H]galactose have been described,[14] using the now readily available methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside as shown in Scheme 1; the tritiated compounds were



Reagents: i, DMSO–Ac_2O; ii, $NaBT_4$

Scheme 1

then subjected to acid hydrolysis. By a series of enzymic procedures involving phosphate esters, D-glucose has been converted into D-[4,5,6-$^3H_3$]-fructose.[14a] A method for determining the extent of ^{14}C-labelling of C-1, C-2, C-3, and C-6 positions in D-glucose has been described.[15] Tritiation of 2-deoxy-D-*erythro*-pentose has been achieved by exposing its tri-*O*-benzoyl derivative to tritium gas in the presence of palladium on charcoal. Hydrolysis gave the active free sugar.[16]

Physical Measurements

The first part of a two-part review on the mutarotation of sugars in solution has appeared,[17] which covers the history, basic kinetics, and composition of sugar solutions.

[10] S. David and M.-O. Popot, *Carbohydrate Res.*, 1968, **8**, 350.
[11] J. W. Mayo and R. L. Anderson, *Carbohydrate Res.*, 1968, **8**, 344.
[12] S. Levine, R. G. Hansen, and H. M. Sell, *Carbohydrate Res.*, 1968, **6**, 382.
[13] L. Gatzsche and H. Haenel, *Ernährungsforschung*, 1967, **12**, 641 (*Chem. Abs.*, 1968, **68**, 78517x).
[14] O. Gabriel, *Carbohydrate Res.*, 1968, **6**, 319.
[14a] C. Jochmann, P. Rauschenbach, and W. Lamprecht, *Z. Physiol. Chem.*, 1968, **349**, 885.
[15] J. C. Turner, *J. Labelled Compounds*, 1967, **3**, 217.
[16] V. M. Vdovenko, V. N. Bobrova, L. S. Gordeeva, V. K. Dedova, A. V. Zharkov, and V. G. Seleznev, *Radiokhimiya*, 1967, **9**, 673.
[17] W. Pigman and H. S. Isbell, *Adv. Carbohydrate Chem.*, 1968, **23**, 11.

The mutarotation of D-glucose has been examined in various solvents by g.l.c. of TMS derivatives. It proceeded at minimal rates in DMF and DMSO, but at measurable rates in pyridine and formamide.[18] It has also been shown[19] that reducing sugars are very slow to attain optical equilibrium in quinoline solution, many weeks generally being necessary.

The equilibrium mixture of D-galactose isomers after mutarotation in pyridine has been investigated by trimethylsilylation (isolation of the products by preparative g.l.c.) and n.m.r. studies. The percentage composition of the mixture was α-furanose (5·1), β-furanose (12·1), α-pyranose (33·8), and β-pyranose (49%).[20] As expected, this mixture differed markedly from that obtained after mutarotation in water.

It is most apt to consider papers on the mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucose in this part of the Report, even though it is not a free sugar. Its mutarotation in benzene, catalysed by carboxylic acids, was shown to be a bifunctional process.[21] When an equimolar mixture of 2,4-dinitrophenol and triethylamine was used in the same solvent, the results suggested that catalysis was by an ion-pair, whose activity was of the order of that of 2-hydroxypyridine.[22] An independent study used benzoic acid and 2-hydroxypyridine as bifunctional catalysts in benzene; thermodynamic parameters were determined and the process was considered to be an enzyme model. In particular, a non-aqueous solvent was considered to simulate more closely the environment of an enzyme active site than the more usually used aqueous systems.[23]

A most important paper has appeared from Angyal on the conformation of free sugars in aqueous solution.[24] Earlier calculations have been refined, to take into account non-bonded interaction energies and the anomeric effect (shown to vary according to the orientation of the group at C-2). Despite the approximations, which it is stressed are inherent in such an approach, the correct chair conformations (determined by n.m.r.) of the α- and β- forms of the aldoses were predicted. Furthermore, the equilibrium α : β ratio for each sugar was calculated and the results were found to be in remarkably good agreement with experimental values. In a further paper, the equilibrium between aldohexoses and their 1,6-anhydrides and between heptuloses and their 2,7-anhydrides was similarly considered (see p. 35).

A simple LCAO–MO method of inductive parameters has been applied to the electronic structure of aldopentoses and the results obtained have been used to interpret their chemical properties.[25]

[18] H. Jacin, J. M. Slanski, and R. J. Moshy, *J. Chromatog.*, 1968, **37**, 103.
[19] A. de Grandchamp-Chaudun, *Ann. pharm. françaises*, 1968, **26**, 115.
[20] T. E. Acree, R. S. Shallenberger, and L. R. Mattick, *Carbohydrate Res.*, 1968, **6**, 498.
[21] A. Kergomard and M. Renard, *Tetrahedron*, 1968, **24**, 6643.
[22] A. Kergomard and M. Renard, *Tetrahedron Letters*, 1968, 769.
[23] P. R. Rony, *J. Amer. Chem. Soc.*, 1968, **90**, 2824.
[24] S. J. Angyal, *Austral. J. Chem.*, 1968, **21**, 2737.
[25] Yu. A. Zhdanov, V. I. Minkin, Yu. A. Ostroumov, and G. N. Dorofeenko, *Carbohydrate Res.*, 1968, **7**, 156.

The ionisation constants of D-glucose, D-fructose, lactose, maltose, and sucrose have been determined by titrimetric methods.[26] The o.r.d. spectra of several tetruloses and 2- and 3-pentuloses have been investigated (see p. 205); c.d. spectra of free sugars have been recorded (see p. 204).

Reactions

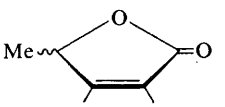
Three papers have appeared on the development of an industrial process for the conversion of D-glucose into D-fructose, catalysed by alkali-metal cations.[27-29] The equilibrium of D-fructose with its 1,2-ene-diol has been studied by deuterium exchange.[30] D-Fructose was treated with calcium or sodium deuteroxide in deuterium oxide and with deuteriosulphuric acid in the same solvent. In the former two systems n.m.r. spectra showed that incorporation of deuterium occurred only at C-1; no incorporation occurred in the acidic system. These results parallel the relative reactivity of the sugar in basic and acidic solutions.

An investigation of the degradation of D-glucose and of cellobiose in the presence of oxygen at high alkalinities was undertaken as a model system for the reaction of cellulose under the same conditions. The main products from D-glucose were formic, gluconic, glycollic, and glyceric acids (together with nine others); cellobiose, in addition to the above, gave aldobionic, 3,4-dihydroxybutyric, 3-deoxy-pentonic, and glucoisosaccharinic acids.[31] The kinetics of the reversion of D-glucose in the presence of hydrogen chloride have been investigated and the thermodynamic parameters calculated.[32]

The pyrolysis of a large variety of carbohydrate materials has been investigated and the products analysed by the powerful g.l.c.–mass spectrometry technique.[33] Aldoses, ketoses, glucono-3,6-lactone, disaccharides, amylose, amylopectin, and cellulose all gave the same products, which led to the suggestion that all these compounds polymerised to form very similar high molecular weight intermediates, which then underwent thermal degradation. The product distribution from glyceraldehyde, 1,6-anhydro-aldohexoses, and alditols was different from that from the above compounds. Addition of basic salts suppressed the formation of furan derivatives and favoured the production of carbonyl compounds. In an independent study,[34] D-glucose was pyrolysed at 250° for 30 min. to give a mixture of more than 100 components investigated by g.l.c.–mass spectrometry. Amongst the products were the previously uncharacterised compounds

[26] S. Z. Ivanov and E. S. Ligin, *Zhur. priklad. Khim.*, 1968, **41**, 2722.
[27] K. Kainuma, K. Tadokoro, and S. Suzuki, *J. Agric. Chem. Soc. Japan*, 1968, **42**, 173.
[28] K. Kainuma, K. Yamamoto, and S. Suzuki, *J. Agric. Chem. Soc. Japan*, 1968, **42**, 243.
[29] K. Kainuma, K. Yamamoto, K. Tadokoro, and S. Suzuki, *J. Agric. Chem. Soc. Japan*, 1968, **42**, 249.
[30] M. S. Feather, *Carbohydrate Res.*, 1968, **7**, 86.
[31] O. Samuelson and L. Thede, *Acta Chem. Scand.*, 1968, **22**, 1913.
[32] J. Hollo, M. Toth, and E. Laszlo, *Stärke*, 1967, **19**, 316.
[33] K. Heyns and M. Klier, *Carbohydrate Res.*, 1968, **6**, 436.
[34] R. H. Walter and I. S. Fagerson, *J. Food Sci.*, 1968, **33**, 294.

(2)–(5). The thermal degradation of D-glucose in aqueous solution at



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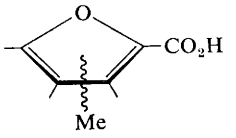

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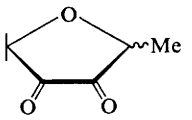

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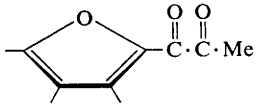


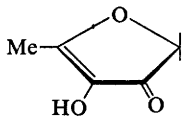
(5)

200—300° has been studied, and the reaction, although complex, was kinetically analysed.[35]

Pyrolysis of D-glucose, D-fructose, and D-xylose gave the 3-deoxyglycosones, isolated as their bis-(3,5-dinitrophenyl)hydrazones, as well as 5-hydroxymethylfurfural and furfural, again isolated in the same way. A mechanism was proposed in which the 3-deoxyglycosones were intermediates in the formation of furfural and its derivatives.[36]

The oxidative degradation of D-glucose to tartaric acid with nitric acid in the presence of a vanadate catalyst was re-evaluated and the product shown to be L-threaric acid (*d*-tartaric acid);[37] this observation conflicts with previous reports. Irradiation of aqueous solutions of D-glucose gave malondialdehyde, the yield of which was studied as a function of the oxygen present.[38]

The reaction of aldopentoses with secondary amine salts has been studied and conditions arranged to give optimal yields (20%) of compound (6)[39] (*cf.* vol. 1, p. 9).



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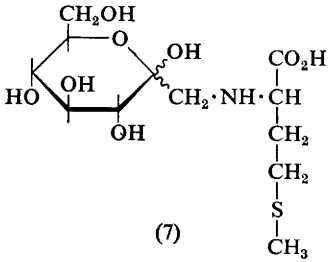


(7)

[35] O. Bobleter and G. Pape, *Monatsh.*, 1968, **99**, 1560.
[36] K. Kato and H. Komorita, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 715.
[37] F. Lesquibe, *Ind. Aliment. Agr. (Paris)*, 1967, **84**, 1493.
[38] H. Scherz and G. Stehlik, *Monatsh.*, 1968, **99**, 1143.
[39] H. G. Peer, G. A. M. van den Ouweland, and C. N. de Groot, *Rec. Trav. Chim.*, 1968, **87**, 1011.

To test a hypothesis that methionine in food proteins reacts with carbohydrates to give compounds that are nutritionally inferior to methionine itself, D-glucose and methionine were allowed to react to give a light brown amorphous powder, which was biologically inferior to the free amino-acid. On the basis of qualitative tests, the product was assigned structure (7).[40] The formation of *N*-substituted pyrolle-2-aldehydes in the browning reaction between D-xylose and various amino-acids has been investigated,[41] the products being isolated as the methyl esters of the 2,4-dinitrophenylhydrazine derivatives.

The ammonolysis of D-glucose and of D-xylose was found to give epimerisation with simultaneous formation of glycosylamines and imidazole derivatives.[42]

The colour produced by reaction of D-glucose, D-fructose, or 5-hydroxymethylfurfural with anthrone in the presence of sulphuric and acetic acids has been shown to be due to 10-[5-(anthron-10-ylmethyl)-2-furfurylidene]-anthrone together with the brown resins formed.[43] Reaction of D-glucose with fumaric acid and butan-1,3-diol polyester in dry hydrogen fluoride gave glucose-containing polymers formed by ester exchange; additions to double bonds occurred to a small extent. With fumaric acid alone, D-glucose gave a three-dimensional cross-linked polymer, which showed marked swelling properties in water.[44]

The influence of carbohydrates on the inhibition of iron(III) oxide sol formation has been studied; sorbitol was more efficient than D-fructose, which in turn was more efficient than D-glucose.[45]

The synthesis of a series of annulene polyoxides from sucrose has been reported.[46] Investigations have also been carried out on the protonic conductivity along lattice defects in sucrose[47] and on the relationship of the sweetness of this carbohydrate and concentration.[48]

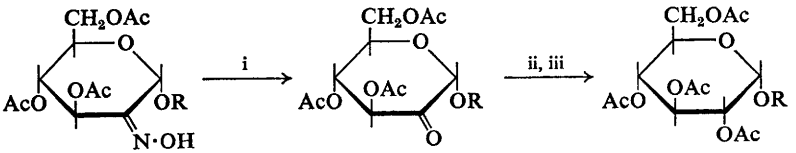
[40] M. J. Horn, H. Lichtenstein, and M. Womack, *J. Agric. Food Chem.*, 1968, **16**, 741.
[41] H. Kato and M. Fujimaki, *J. Food Sci.*, 1968, **33**, 445.
[42] M. S. Dudkin, N. G. Shkantova, and A. F. Yatsuk, *Zhur. priklad. Khim.*, 1968, **41**, 385.
[43] H. Hoermann and I. R. Siddiqui, *Annalen*, 1968, **714**, 174.
[44] F. Micheel and M. Buller, *Chem. Ber.*, 1968, **101**, 3729.
[45] T. Fujita, *J. Chem. Soc. Japan*, 1968, **89**, 343.
[46] J. A. Elix, *Chem. Comm.*, 1968, 343.
[47] J. M. Thomas and J. O. Williams, *Chem. Comm.*, 1968, 209.
[48] J. Gordon, *J. Food Sci.*, 1968, **33**, 483.

3
Glycosides

O-Glycosides

Synthesis.—Several papers of significance in this area were published in 1968; in particular an important new α-glucopyranoside synthesis was described, and the orthoester route to 1,2-*trans*-glycosides was developed further. In addition, specific syntheses of unsaturated glycosides (which on hydroxylation give normal glycosides) and of benzyl glycosides were reported, and new information has been gained on the methanolysis of simple sugars. For the first time dialkyl acetals were detected, but these were found not to be important intermediates in the reactions.

From Lemieux's laboratory an important development in the synthesis of α-D-glucopyranosides was reported which appears to have wide general potential. Treatment of 3,4,6-tri-*O*-acetyl-2-oximo-α-D-*arabino*-hexopyranosides, which are readily obtainable from tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucosyl chloride (see p. 84), with a carbonyl compound (levulinic acid used) in the presence of hydrochloric acid, caused deoximation and the liberation of the keto-derivatives. These on reduction with sodium borohydride gave the glucosides with high (>90%) specificity (Scheme 2).[49]



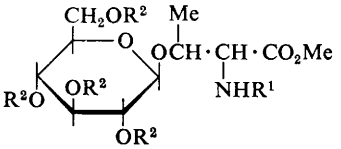
Reagents: i, $R_2^1CO-H^+$; ii, $NaBH_4$; iii, Ac_2O

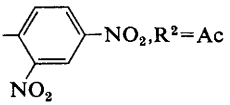
Scheme 2

1,2-*trans*-Glycosides can be synthesised by way of 1,2-orthoesters and the reaction has been reviewed by Kochetkov.[50] A further application has been to the arabinitol galactoside, umbilicin, found in lichens, which has thus been shown to be 2-*O*-β-D-galactofuranosyl-D-arabinitol.[51]

[49] R. U. Lemieux, R. Suemitsu, and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 1040.
[50] N. K. Kochetkov, *Kem. Kozlem.*, 1967, **28**, 425.
[51] H. F. G. Beving, H. S. Boren, and P. J. Garegg, *Acta Chem. Scand.*, 1968, **22**, 193.

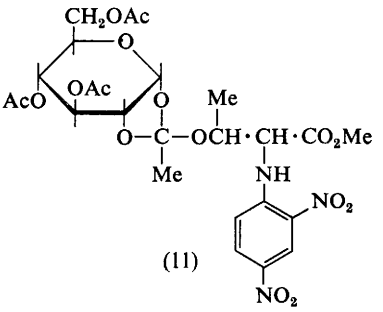
Glycosides may also be synthesised from 1,2-orthoesters by an intramolecular rearrangement: thus the threonine derivative (8) was prepared



(8) R^1=—  —NO_2, R^2=Ac

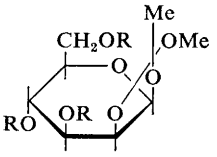
(9) R^1= H= R^2

(10) R^1= CO_2CH_2Ph, R^2= Ac

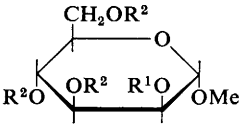


(11)

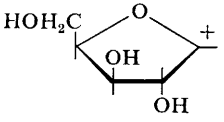
via (11) and converted into (9). Similarly, the *N*-benzyloxycarbonyl compound (10) was prepared and converted to (9). Compounds (8) and (10) showed acid stability comparable to that of alkyl glycosides, but the orthoesters were extremely acid-labile.[52] Parallel work has been accomplished in the β-D-mannopyranose series, the main objective, however, being the synthesis of 2-*O*-substituted mannoses. Benzylation of (12) gave



(12) R= Ac
(13) R= CH_2Ph



(14) R^1 = H, R^2 = CH_2Ph
(15) R^1 = Me, R^2 = CH_2Ph
(16) R^1 = Ac, R^2 = CH_2Ph



(17)

(13) which with methanolic hydrogen chloride gave methyl 3,4,6-tri-*O*-benzyl-α-D-mannoside (14) (>90%). Methylation of (14) gave the 2-*O*-methyl ether (15) which on catalytic hydrogenolysis afforded methyl 2-*O*-methyl-α-D-mannopyranoside. Alternatively, methanolysis of (13) with toluene-*p*-sulphonic acid or with mercury(II) bromide yielded mainly (16); similar rearrangements were observed with the isopropyl and cyclohexyl orthoacetates.[53]

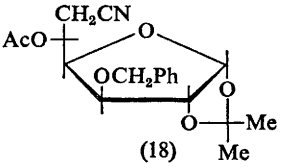
[52] V. A. Derevitskaya, E. M. Klimov, and N. K. Kochetkov, *Carbohydrate Res.*, 1968, **7**, 7.

[53] N. E. Franks and R. Montgomery, *Carbohydrate Res.*, 1968, **6**, 286.

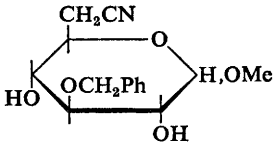
Two papers have described the application of radiochemical methods to the study of the methanolyses of free sugars. It was shown (^{14}C counting of chromatograms) that the hitherto undetected methyl acetals of D-xylose and D-glucose were formed, but that their concentrations did not at any time exceed 2·5%. The results indicated that the acetals were not primary products but that the hemiacetals may well have been. The initial step in the reaction was then discussed, and because the α-furanosides were the main kinetic products the ion (17) was considered an unlikely intermediate. Similarly, it was thought unlikely that direct substitution at C-1 of the β-furanoses occurred at the outset.[54] In a related study, published at approximately the same time, the relative rates of methyl glycoside formation from the four pentoses were found to be similar to those of 1,4-anhydride formation from pentitols, indicating that an acyclic intermediate may have been involved in the glycosidation process. With the aid of ^{14}C-labelled arabinose and galactose and by application of kinetic methods (dilution analysis) it was shown that such intermediates were not the dimethyl acetals but hemiacetals. The small amounts of dimethyl acetals which were formed were produced either from the furanosides or concurrently with them.[55] Following their work on the detailed investigation of the methanolyses of the pentoses using g.l.c. methods, Bishop and his co-workers have now examined the reactions of glucose, galactose and mannose. They found, as before, that the processes can be divided into, (i) the formation of furanosides (no acetals were observed by these methods), (ii) the anomerisation of the furanosides, (iii) the furanoside to pyranoside interconversion, and (iv) the final equilibration of the pyranosides. The findings at each stage were rationalised in terms of the relative conformational stabilities of the species involved.[56]

Methanolyses of some sugar derivatives have been studied. From 2-deoxy-D-*ribo*-hexose, under mild conditions, the furanosides were obtained exclusively and in equal proportions,[57] and in a reinvestigation of 2-deoxy-D-*arabino*-hexose it was shown[58] that when 35% of the starting material remained, the products consisted of 30% pyranosides (α, β; 1 : 1) and 35% methyl 2-deoxy-α-D-*arabino*-hexofuranoside which was obtained crystalline. This five-membered compound was then used in nucleoside syntheses. Similar reaction of compound (18), prepared from the 5-hydroxy-6-*O*-toluene-*p*-sulphonyl analogue by treatment with potassium cyanide in methanol followed by acetylation, gave the pyranoside (19) and the furanoside (20) which were isolated by chromatographic procedures and examined by n.m.r. spectroscopy, the latter technique showed that the mechanical rotation of the benzyl group was subject to

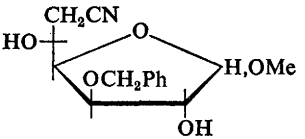
[54] R. J. Ferrier and L. R. Hatton, *Carbohydrate Res.*, 1968, **6**, 75.
[55] D. D. Heard and R. Barker, *J. Org. Chem.*, 1968, **33**, 740.
[56] V. Smirnyagin and C. T. Bishop, *Canad. J. Chem.*, 1968, **46**, 3085.
[57] C. C. Bhat, K. V. Bhat, and W. W. Zorbach, *Chem. Comm.*, 1968, 808.
[58] K. V. Bhat and W. W. Zorbach, *Carbohydrate Res.*, 1968, **6**, 63.



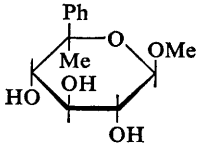
(18)



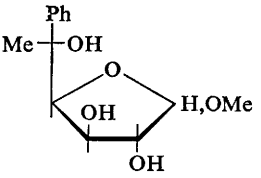
(19)



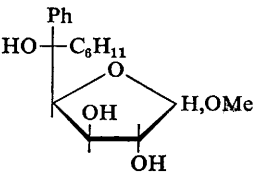
(20)



(21)



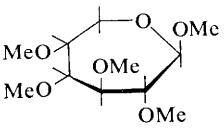
(22)



(23)

restriction.[58a] Similarly, 6-deoxy-5-*C*-phenyl-L-idose gave the α-pyranoside (21) and the two furanosides (22); 5(*R*)-5-*C*-cyclohexyl-5-*C*-phenyl-D-xylose, however, gave only the two furanosides (23).[59] The structures of these products were established by periodate oxidation and by n.m.r. analysis, the latter technique being assessed for its use in assigning furanose ring conformations. The results show that pyranose derivatives having large *syn*-axial substituents at C-1 and C-5 are not formed.

Of special interest was the report of the formation of the methyl β-D-glucoseptanoside ether (24), the only glucoseptanose derivative known, by

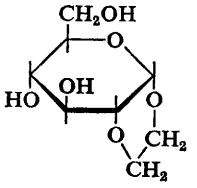


(24)

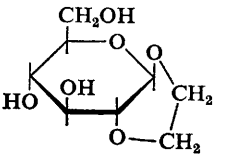
methanolysis, *via* the acetal of 2,3,4,5-tetra-*O*-methyl-D-glucose.[60] Methanolysis of 3-amino-3,6-dideoxy-1,2-*O*-isopropylidene-α-D-allofuranose gave the anomeric pyranosides illustrating that a ring expansion occurred during the reaction.[61]

[58a] I. Dijong and U. Wittkötter, *Chem. Ber.*, 1968, **101**, 1948.
[59] T. D. Inch and P. Rich, *J. Chem. Soc.* (*C*), 1968, 1784.
[60] E. F. L. J. Anet, *Carbohydrate Res.*, 1968, **8**, 164.
[61] J. Kovář and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 626.

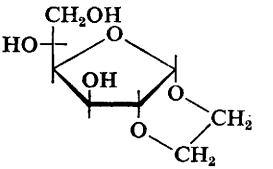
In an interesting, related investigation, 2-*O*-hydroxyethyl-D-glucose was treated with hydrogen chloride in DMF and gave a mixture of products, two of which were shown to be the bicyclic pyranosides (25) and (26). The



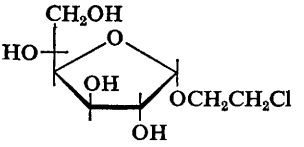
(25)



(26)



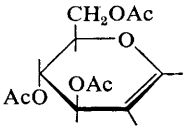
(27)



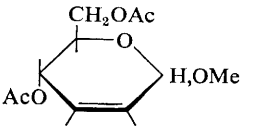
(28)

main product, however, was a furanoside which had the α-configuration (27) despite its negative optical rotation ($[\alpha]_D$ −56°). The furanoside : pyranoside ratio was 2·5 : 1 and the α-pyranoside was formed in larger quantities than its anomer. D-Glucose on direct treatment with 2-chloroethanol yielded a mixture of 2-chloroethyl glycosides from which the pyranosides and the α-furanoside (28) were obtained after fractionation on a column of silica gel. This last compound with ethanolic sodium hydroxide cyclised to give compound (27) in good yield.[62]

A specific method for the synthesis of 2,3-unsaturated glycosides is exemplified by the reaction of tri-*O*-acetyl-D-glucal (29) with methanol in the presence of boron trifluoride. The reaction goes to completion and the α- and β-products (30) are formed in the ratio 10 : 1. The reaction can be applied in inert solvents with equimolar proportions of complex alcohols as shown by the preparation of the disaccharide derivative (31) (50%).



(29)



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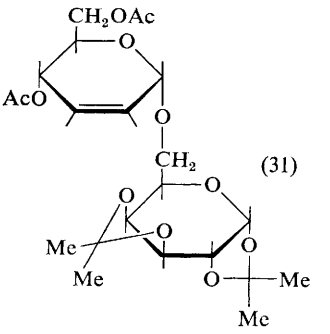
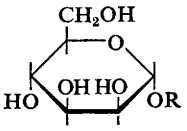


(31)

[62] J. E. Höök and B. Lindberg, *Acta Chem. Scand.*, 1968, **22**, 2157.

Permanganate hydroxylation followed by removal of the protecting groups gave 6-*O*-(α-D-mannopyranosyl)-α-D-galactose (32). Alternatively, epoxidation of (31) gave a crystalline epoxide which, after alkaline hydrolysis and removal of the protecting groups, yielded (33) and (34).[63]



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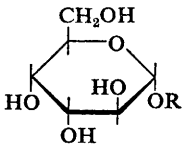
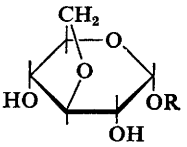
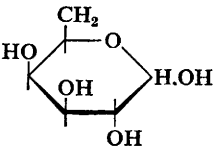


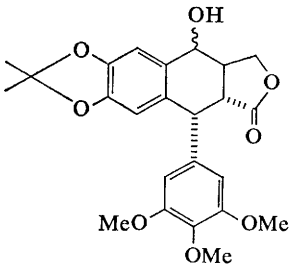
(33)



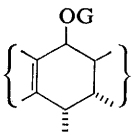
(34)

R= 

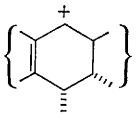
A method for the synthesis of benzylic glycosides has been described which apparently involved substitution by a glycosyloxy rather than the usual glycosyl species. Treatment of the epimeric compounds (35) with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in the presence of boron trifluoride



(35)



(36)



(37)

$GOCH(Ph)_2$

(38)

G = tetra-*O*-acetyl-β-D-glucopyranosyl

at low temperatures gave the acetylated β-glycoside (36) as the main product. It was concluded that the reaction proceeded by alcohol attack on the carbonium ion (37) from the less-hindered side; in similar fashion, benzhydrol gave (38).[64] Unfortunately, it seems that the reaction will be applicable only to the glycosylation of alcohols giving stable carbonium ions of the benzyl type.

The chemistry of phenyl glycosides has been reviewed;[65] o.r.d. spectra of compounds of this series are referred to on p. 204.

[63] R. J. Ferrier and N. Prasad, *Chem. Comm.*, 1968, 476.
[64] M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, 1968, **51**, 1631.
[65] M. Psenak, *Biologia*, 1967, **22**, 704; *Chem. Abs.*, 1968, **68**, 39958x.

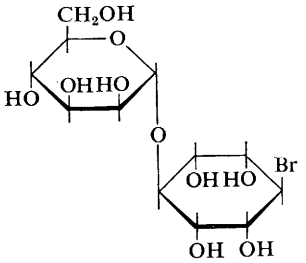
Glycosyl transferase activity has been further examined. In one investigation, transfer was shown to occur in the presence of a β-thioglucosidase (from black mustard) from *p*-nitrophenyl β-D-glucopyranoside to glycerol to give 1-*O*-(β-D-glucopyranosyl)-glycerol. The reaction, therefore, proceeds with retention of configuration.[66] Similarly, an enzyme isolated from *Clostridium thermocellum* has been shown to transfer the glucose units of α-D-glucose 1-phosphate to the 4-position of other sugars, but with inversion of configuration. In this way cellobiose, 4-*O*-β-D-glucopyranosyl-2-deoxy-D-glucose, -D-mannose, -2-amino-2-deoxy-D-glucose, -D-xylose, and -D-arabinose have been prepared.[67]

A specific synthesis of methyl β-D-lyxofuranoside, which cannot be prepared by methanolysis of the free sugar, has been described (see p. 73). Syntheses of methyl α- and β-D-allopyranoside were reported by routes closely related to those previously described (vol. 1, p. 69), the β-isomer being obtainable from the anomeric mixture formed from the α-form.[68] Other specific glycoside derivatives were prepared from 1,2-*O*-isopropylidene-α-D-glucofuranose. Alkyl 3,5,6-tri-*O*-benzyl-D-glucofuranosides (and their 2-esters) thus obtained were found to be pharmacologically active. The anomerisations of the compounds and their conformations as revealed by n.m.r. spectroscopy were investigated and discussed.[69]

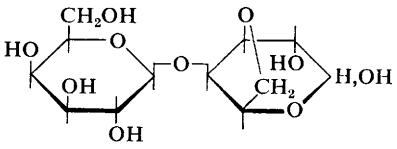
The Koenigs–Knorr reaction continues to hold the most important place amongst synthetic methods applicable to complex glycosides. The preparation of deoxyglycosides *via* *O*-acyldeoxyglycosyl halides has been surveyed,[70] and the method has been applied to a variety of compounds: 7-(β-D-glucopyranosyloxy)-derivatives of 3-coumarins,[71] 2-aminoethyl β-D-glucopyranoside (prepared to compare the effect of the amino-group in this position with that at C-2 in 2-amino-2-deoxy-D-glucose on the acidic hydrolysis of the glycoside (see p. 22),[72] and a cyclitol α-D-mannopyranoside (39).[73] The notable feature of this last synthesis was the quantitative yield—quite unusual for a condensation of this type. The α-mannopyranosides of the isomeric 2-aminocyclohexanols were similarly prepared during the investigation.[74]

Disaccharides which have been made by the Koenigs–Knorr synthesis include the α- and β-isomers of 2-*O*-(D-glucopyranosyl)-D-glucose,[75] prepared in acetonitrile solution in the presence of mercury(II) bromide and

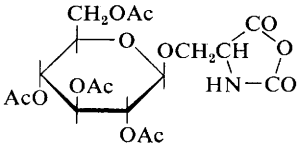
[66] G. A. Howard and R. D. Gaines, *Phytochemistry*, 1968, **7**, 585.
[67] J. K. Alexander, *Arch. Biochem. Biophys.*, 1968, **123**, 240.
[68] J. S. Brimacombe and A. Husain, *Carbohydrate Res.*, 1968, **6**, 491.
[69] G. Huber and A. Rossi, *Helv. Chim. Acta*, 1968, **51**, 1185.
[70] W. W. Zorbach, C. C. Bhat, and K. V. Bhat, *Adv. Chem. Ser.*, 1968 (No. 74), 1.
[71] W. R. Sherman and E. Robins, *Carbohydrate Res.*, 1968, **7**, 184.
[72] E. R. B. Grahan and A. Neuberger, *J. Chem. Soc.* (*C*), 1968, 1638.
[73] H. Shibata, D. Nishimura, N. Kurihara, and M. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1968, **32**, 1002.
[74] N. Kurihara, T. Ueno, S. Hashimoto, and M. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 1343.
[75] B. H. Koeppen, *Carbohydrate Res.*, 1968, **7**, 410.



(39)



(40)



(41)

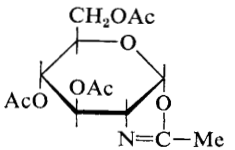
cyanide (conditions which are known to lead to mixed products), and agarobiose (40) [3,6-anhydro-4-*O*-(β-D-galactopyranosyl)-L-galactose][76] which is also obtained by partial hydrolysis of agar. In addition, the syntheses of methyl and benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside have been carried out (using hepta-acetylchitobiosyl chloride) for application of the products in lysozyme studies.[77] Other disaccharide derivatives which have been synthesised are the four possible *O*-β-D-glucopyranosyl-(1 → *n*)-*O*-β-D-glucopyranosyl-(1 → 1)-D-glycerols (*n* = 2, 3, 4, or 6) and 1-*O*-α-D-kojibiosyl-L-glycerol; these were compared chromatographically with compounds of natural origin.[78] Furthermore, the *O*-L-serine β-glycosides of D-galactose, lactose, cellobiose, 2-acetamido-, and 2-dodecamido-D-glucose and the α-L-rhamnosyl analogue were prepared using *N*-benzoyloxycarbonyl and benzyl ester protecting groups. The *O*-acetylated derivatives were then treated with phosgene to give *N*-carboxy-α-amino acid anhydrides, *e.g.* (41) useful in the synthesis of sugar–peptide linkages.[79]

Appreciable developments have taken place in the synthesis of glycosides of 2-amino-2-deoxy sugars. In particular, Lemieux and his co-workers have utilised the tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glycopyranosyl chlorides obtained in excellent yield on the addition of nitrosyl chloride to tri-*O*-acetylglycals. These have been shown to react readily with alcohols in DMF at room temperature to give tri-*O*-acetyl-2-oximino-α-D-hexopyranosides[80] (see p. 84) which can then be reduced to 2-amino-2-deoxy glycosides.

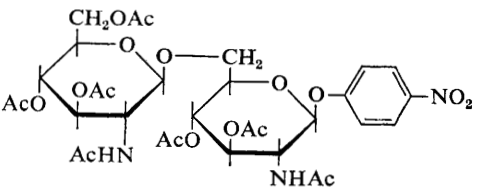
A method, analogous to the orthoester glycoside method (p. 11), for preparing 1,2-*trans*-glycosaminides has been given some attention: various

[76] S. Hirase, C. Araki, and K. Arai, *Bull. Chem. Soc. Japan*, 1968, **41**, 626.
[77] U. Zehavi and R. W. Jeanloz, *Carbohydrate Res.*, 1968, **6**, 129.
[78] D. E. Brundish and J. Baddiley, *Carbohydrate Res.*, 1968, **8**, 308.
[79] E. Rüde and M. Meyer-Delius, *Carbohydrate Res.*, 1968, **8**, 219.
[80] R. U. Lemieux, T. L. Nagabhushan, and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 405.

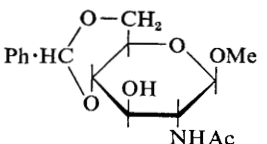
1,2-oxazoline compounds for example (42) were prepared from amino-sugar derivatives and were shown to be convenient glycosylating agents as



(42)



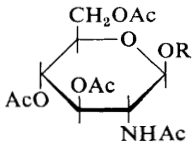
(43)



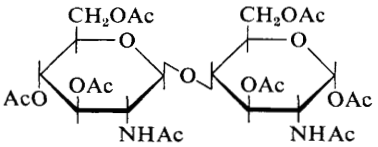
(44)

was illustrated by the synthesis (in nitromethane using toluene-*p*-sulphonic acid as catalyst) of (43) from the appropriate α-oxazoline and *p*-nitrophenyl 3,4-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside.[81] In related work the same group prepared the β-glycoside (44) by methanolysis of the corresponding oxazoline, and also obtained the β-mannose oxazoline, which gave the α-mannosaminide but at an appreciably slower rate. The α-*gluco*-phenyloxazoline was much more reactive towards methanolysis than was the methyl compound.[82]

American workers have also examined this reaction with the ingenious aim of developing a method for the selective alcoholysis of glycosaminide bonds in polysaccharides (2-acylamino-groups participating in the cleavage of *trans* glycosidic bonds more readily than do 2-acyloxy groups). In model experiments compounds (45) and (46) readily gave the benzyl



(45) R = Ac
(46) R = Me
(47) R = CH_2Ph



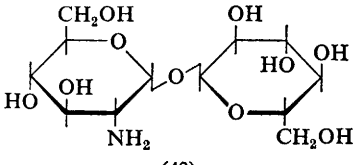
(48)

glycoside (47) on heating in the presence of zinc chloride and benzyl

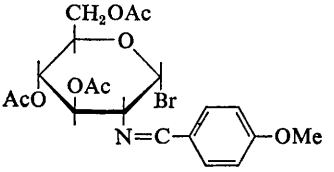
[81] Ya. A. Khorlin, M. L. Shul'man, S. E. Zurabyan, I. M. Privalova, and Yu. L. Kopaevich, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 227.
[82] M. L. Shul'man, I. M. Privalova, and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2655.

alcohol, whereas the α-anomers and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside reacted slowly if at all. Unfortunately the disaccharide derivative (48) also reacted only slowly.[83]

The difficulties encountered in the synthesis of 2-amino-2-deoxyglycosides which result from the rearrangement of 2-acetamido-2-deoxyglycosyl halides into 2-amino-2-deoxyglycosyl acetate hydrohalides have also been overcome by use of the dichloroacetamido group; the method has been applied to the synthesis of 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose,[84] a component of gangliosides. Trehalosamine (49)



(49)

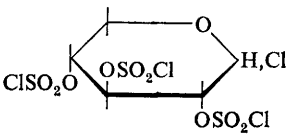


(50)

which has a strong antibacterial activity against *Mycobacterium tuberculosis* was prepared using the *p*-anisylidene group as a blocking group, *i.e.* condensing (50) with 2,3,4,6-tetra-*O*-benzyl-D-glucose, followed by removal of the blocking groups.[85]

6-Acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-D-glucopyranosyl chloride, prepared from methyl 6-azido-2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranoside, has been condensed with *N*-substituted (+)- or (−)-2-aminocyclohexanol to give a single product in each case (believed to have the α-configuration) in a reaction designed as a model for antibiotic synthesis.[86]

Compounds (51) prepared by the reaction of D-xylose with sulphuryl chloride (see p. 59) each underwent methanolysis to give methyl glycosides



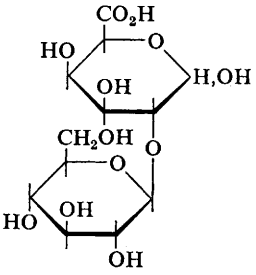
(51)

of the opposite configuration; the α-compound reacted more slowly than the β (which may indicate that the latter reacts in the D-1*C* conformation). In a disaccharide synthesis the β-chloride afforded a satisfactory route to 6-*O*-α-D-xylopyranosyl-D-mannose, but the α-compound was too unreactive to be used in the preparation of the corresponding β-disaccharide. This

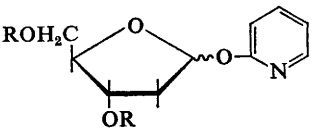
[83] W. L. Salo and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 3585.
[84] A. J. Acher and D. Shapiro, *Israel J. Chem.*, 1967, **5**, 61P.
[85] S. Umezawa, K. Tatsuta, and R. Muto, *J. Antibiotics (Japan), Ser. A*, 1967, **20**, 388.
[86] T. Ueno, N. Kurihara, S. Hashimoto, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 1346.

was prepared from 1,2,3,4-tetra-*O*-acetyl-6-*O*-triphenylmethyl-α-D-mannopyranose which was condensed with tetra-*O*-acetyl-α-D-xylopyranosyl bromide.[87]

Uronosides of steroids,[88] and of codeine and morphine [89] have been synthesised (by the Koenigs–Knorr method); a *pseudo*-aldobiouronic (52) was obtained together with smaller proportions of the α-isomer by appropriate condensations.[90]



(52)



(53)

R = *p*-toluoyl

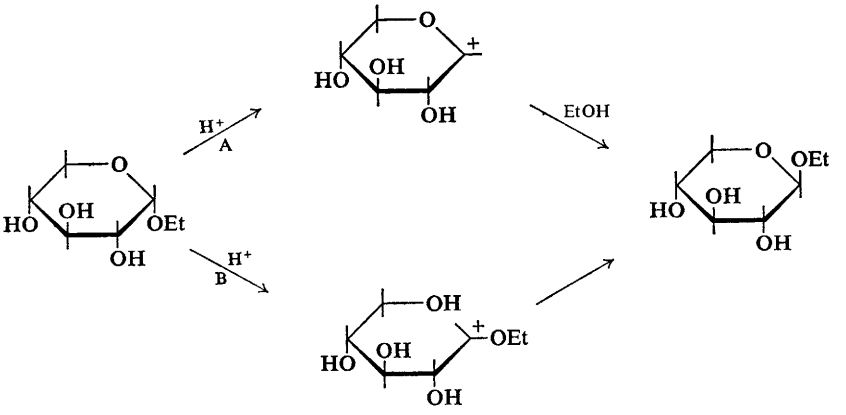
Reaction of a variety of substituted 2-hydroxypyridines with acetobromoglucose gave both *O*- and *N*-linked glycosides in accordance with previous findings of the authors and others.[91] This report is only one of several by Wagner *et al.* on the glycosylation of heterocyclic compounds; other reference is omitted because the main interest was in the simple preparation of glycosylated products of potential pharmacological value. The same authors studied the anomerisation and *O*- to *N*- rearrangement of 2-(tetra-*O*-acetyl-β-D-glucopyranosyloxy)-pyridines catalysed by mercury(II) bromide in toluene,[92] and they also studied the *O*- to *N*- migration of compounds (53, α- and β-anomers). Both *N*-linked pyridone anomeric products were obtained from each starting material.[93] Other related studies on this topic by the same authors have been published.[94–96]

The kinetics of the reversion of glucose in the presence of hydrochloric acid were investigated and thermodynamic parameters were reported.[32]

Hydrolysis and Anomerisation.—Reference has been made to the anomerisation of glycosides in the preceding paragraphs. More specifically, the mechanism of anomerisation of the ethyl D-xylopyranosides was investigated using a technique involving two sets of parallel experiments, one of

[87] H. J. Jennings, *Canad. J. Chem.*, 1968, **46**, 2799.
[88] R. Emilozzi, *Bull. Chem. Soc. France.*
[89] H. Yoshimura, K. Oguri, and H. Tsukamoto, *Tetrahedron Letters*, 1968, 483.
[90] P. Šipoš and Š. Bauer, *Carbohydrate Res.*, 1968, **6**, 494.
[91] G. Wagner and H. Gentzsch, *Arch. Pharm.*, 1968, **301**, 201.
[92] G. Wagner and H. Gentzsch, *Arch. Pharm.*, 1968, **301**, 346.
[93] D. Heller and G. Wagner, *Z. Chem.*, 1968, **8**, 415.
[94] G. Wagner and F. Süss, *Die Pharmazie*, 1968, **23**, 8.
[95] H. Kühmstedt and G. Wagner, *Arch. Pharm.*, 1968, **301**, 660.
[96] G. Wagner and H. Gentzsch, *Arch. Pharm.*, 1968, **301**, 346.

which used ^{14}C incorporated in the glycosides, and the other used unlabelled glycoside in radioactive ethanol. In the first cases the total rates of anomerisation were determined; the second gave the rates of incorporation of solvent, and thus the rate of anomerisation which proceeded by a cyclic ion mechanism. It was concluded that the reaction occurred by a cyclic (Scheme 3A) and not an acyclic ion process (Scheme 3B), and that



Scheme 3

displacement of the aglycone proceeded with predominant inversion of configuration. However, with the β-anomer, in particular, appreciable retention of configuration was observed.[97]

Anomerisation of methyl α-D-allopyranoside afforded a means of isolating the β-isomer.[68]

Acidic hydrolysis studies on a variety of glycosides of unsubstituted sugars continue to be reported. Reviews of the literature on the acid hydrolysis of methyl α- and β-D-glucopyranosides have appeared in which thermodynamic data obtained from many sources were correlated.[98, 99] A paper of importance to the basis of this topic has appeared on the hydrolysis of 2-alkoxy- and 2-aroyloxytetrahydropyrans,[100] and a detailed kinetic investigation of the hydrolysis of twenty-seven alkyl β-D-xylopyranosides has been reported. The results were in accord with the expected *A*-1 mechanism.[101]

Cleavage of oligosaccharides with acidic resins has been described and the chromatographic separation of the products from contaminating amino-acids was discussed;[102] in other work, the hydrolysis of sucrose

[97] R. J. Ferrier, L. R. Hatton, and W. G. Overend, *Carbohydrate Res.*, 1968, **8**, 56.
[98] J. Szejtli, *Stärke*, 1967, **19**, 173.
[99] J. Szejtli, *Acta Chim. Acad. Sci. Hung.*, 1968, **56**, 175.
[100] T. H. Fife and L. K. Jao, *J. Amer. Chem. Soc.*, 1968, **90**, 4081.
[101] C. K. de Bruyne and F. van Wijnendaele, *Carbohydrate Res.*, 1968, **6**, 367.
[102] M. Pöhm and E. Silhan, *Mikrochim. Acta*, 1968, 1281.

under the catalytic influence of amino-acids and polyhydroxyphenols was reported.[103] (See p. 23 for further di- and tri-saccharides.)

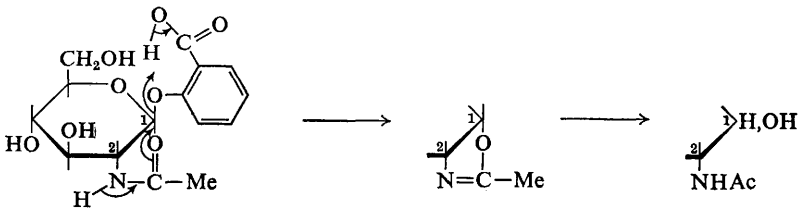
Indolyl β-D-glucopyranosides have been used in histochemical investigations to locate glycosidases since these enzymes liberate indoxyl which rapidly oxidises to indigo. The acid hydrolysis has now been studied kinetically to determine whether the complex aglycone confers any unusual features on the hydrolyses; the colour formation was found to be dependent upon the hydrolysis and not the oxidation step, and the overall kinetic features suggested that the usual *A*-1 mechanism was in operation. However, ΔS^* was only slightly positive which was taken to indicate the possible incursion of other processes.[104]

In a comparative study, the acid hydrolyses of a variety of β-*S*-glucosides and β-*S*-glucuronosides were compared with the reactions of the corresponding *O*-derivatives. The thio-compounds were hydrolysed more slowly than the oxygen analogues with the exception of the 2-hydroxyethyl glucuronoside pair.[105]

Two important papers have appeared on the hydrolysis of 2-acetamido-2-deoxy-D-glucopyranosides which throw light on the possible mode of action of lysozyme. In one, a detailed kinetic investigation of the anomeric methyl compounds was undertaken and the results were compared with those obtained from the simple glucosides. Methyl 2-acetamido-2-deoxy-β-D-glucopyranosides hydrolysed much faster than was anticipated, which led to the conclusion that intramolecular catalysis was occurring by nucleophilic displacement of the protonated aglycone by the amide group. Such catalysis only operated when the aglycone was small enough to allow the ring to distort to give a favourable conformation for participation.[106] In the other paper, the kinetics of hydrolysis of *O*-carboxyphenyl β-D-glucopyranoside and the 2-acetamido-2-deoxy analogue were reported over the pH range 0·75–11·8. At low pH values intramolecular participation of the carboxylic acid group occurred, and, in addition, it was concluded that in the latter compound the 2-acetamido group participated synchronously (Scheme 4). A value of $-\Delta S$ for the amino-glycoside larger than that for the hydroxy analogue is attributed to the necessity in the former for orientating the two catalytic groups correctly in the formation of the transition state.[107] In both these reports the results are considered in relationship to their relevance in the mechanism of 2-acetamido-2-deoxyglycoside hydrolysis by lysozyme. 2-Aminoethyl β-D-glucopyranoside, mentioned earlier,[72] was synthesised to compare the effect of the amino-group in that position with that of one at C-2 on the stability of the glycosidic bond in acidic media. Kinetic studies showed that although the rate was less than

[103] V. A. Afanas'ev and V. I. Gorykova, *Inst. Org. Khim., Akad. Nauk Kirg. S.S.S.R.*, 1967, 19.
[104] J. P. Horwitz, C. V. Easwaran, and L. S. Kowalczyk, *J. Org. Chem.*, 1968, **33**, 3174.
[105] M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, 1968, **6**, 121.
[106] D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1968, **90**, 5844.
[107] D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1968, **90**, 2156.

that of the ethyl glucoside it was much greater than that of methyl 2-amino-2-deoxy-β-D-glucopyranoside.



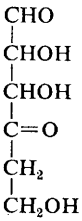
Scheme 4

Several papers have appeared on the stabilities of glycosidic bonds as parts of investigation into the effects influencing the hydrolyses of glycosidic bonds of polysaccharides. Hook and Lindberg have thus examined the eight hydroxyethyl ethers of methyl α- and β-D-glucopyranosides and found that the substituted glycosides were invariably hydrolysed more slowly than the parent compounds, the effect being most apparent when the substituent was at C-6.[108] In related work Timell and co-workers have used di- and tri-saccharide models: 2-*O*-, 6-*O*-, and 6′-*O*-(β-D-glucopyranosyluronic acid)-cellobioses (no cellobiose formed, showing the relative stability of the uronosyl bonds),[109] 2-*O*-, 2′-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-xylobiose and 2′-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-xylobiose (xylobiose was hydrolysed faster than these compounds by factors of 1·2, 7·0, and 3·4 respectively),[110] 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose and 4-*O*-(β-D-glucopyranosyl)-D-glucuronic acid,[111] and related disaccharides.[112] Furthermore, they investigated the hydrolyses of isopropyl and 2-chloroethyl 3-*O*-methyl-β-D-glucopyranosiduronic acids and found them to be less stable than the corresponding unmethylated compounds, a finding which (in keeping with the results reported above [108]) is not consistent with those obtained for the analogous glucopyranosides. A number of 'glucopyranosides' in which the group at C-6 was varied (Me, CH_2OH, CH_2OMe, CH_2Hal, CO_2^-, CO_2H, $CH_2NH_3^+$) were also studied and it was shown that the rates of hydrolysis were inversely proportional to the electron affinity of the C-6 group. The hydrolysis rate of methyl α-D-glucopyranosiduronic acid was calculated on the basis of polar effects to be 15 times less than the observed rate suggesting that anchimeric assistance of the carboxyl group occurred. It was further shown that even in N-sulphuric acid the contribution of the carboxylate ion to the reaction must be considered.[113] Cationic resins can

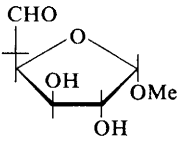
[108] J. E. Höök and B. Lindberg, *Acta Chem. Scand.*, 1968, **22**, 921.
[109] N. Roy and T. E. Timell, *Carbohydrate Res.*, 1968, **6**, 475.
[110] N. Roy and T. E. Timell, *Carbohydrate Res.*, 1968, **6**, 482.
[111] N. Roy and T. E. Timell, *Carbohydrate Res.*, 1968, **7**, 17.
[112] N. Roy and T. E. Timell, *Carbohydrate Res.*, 1968, **6**, 488.
[113] M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, 1968, **6**, 12.

be utilised to cleave glycosidic bonds without causing hydrolysis of acetate groups (see p. 45).

Other Reactions and Features of Glycosides.—One of the main products of radiolysis of methyl α-D-glucopyranoside was shown to be the 5-deoxy-4-ketohexose (54)[114] and another has been characterised by the Russian investigators as the 5-deoxyfuranoid compound (55).[115]



(54)



(55)

The synthesis of several substituted benzoyl esters of methyl glucopyranosides was undertaken to investigate the stability of the glycosidic bond to solid-state radiolysis. These products were found to be stable; however, the tetra-toluene-*p*-sulphonate and the 6-chloro-6-deoxy-2,3,4-tri-toluene-*p*-sulphonate [mistakenly described (see p. 60) as the 4-chlorinated isomer] were affected by the radiation, and an unidentified product absorbing at 1725 cm.$^{-1}$ was detected.[116]

Two papers appeared on the physical properties of phenyl glycosides in connection with their behaviour under biochemical conditions. Aqueous solutions of phenyl β-D-glucopyranoside, *o*-nitrophenyl β-D-galactopyranoside, and 4-methylumbelliferyl β-D-glucopyranoside were found to behave ideally (5×10^{-3} M—saturation) by vapour pressure measurement, and consequently anomalous Michaelis constants noted for such compounds and related glycosides were concluded to be due, improbably, to the non-ideal behaviour of the solutions.[117] Secondly, it was shown, by measuring the partition coefficients between water and octan-1-ol of an extensive series of aryl-substituted phenyl β-D-glucopyranosides, that the 'hydrophobicities' of the substituents were in good agreement with values previously determined for phenoxyacetic acids. Furthermore, the determined values were found to be additive and were independent of the position of the substituent on the aromatic rings. Alteration of the configuration at the anomeric centre influenced the results. The determined hydrophobicities were believed to be related to the binding of such substrates to proteins,

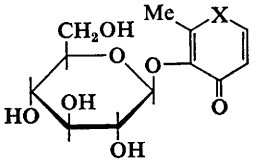
[114] N. K. Kochetkov, L. I. Kudriashov, and M. A. Chlenov, *Zhur. obshchei Khim.*, 1968, **38**, 79.
[115] N. K. Kochetkov, L. I. Kudriashov, M. A. Chlenov, and O. S. Chizhov, *Doklady Akad. Nauk S.S.S.R.*, 1968, **179**, 1385.
[116] I. M. Sarkar and J. C. Arthur jun., *Carbohydrate Res.*, 1968, **6**, 207.
[117] D. H. Leaback, *Biochem. J.*, 1968, **106**, 14P.

and evidence was given briefly of a direct relationship between 'hydrophobicity' values and binding of substituted aryl glycosides to concanavalin A.[118]

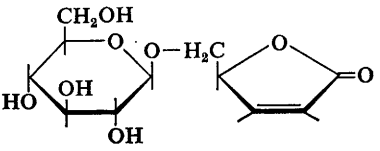
Natural Products.—A vast number of naturally occurring glycosides continue to be reported and our selection represents only the few which are believed to have specific interest from the carbohydrate point of view. Many with simple or complex sugar moieties have not been included.

A detailed n.m.r. study has been made of acetylated digitalis glycosides in an effort to locate the ester groups,[119] and mass spectrometry has been assessed as an aid to structural determination of natural glycosides. All compounds cleaved initially at C-1 so that the aglycones could be partially characterised. The presence of ester groups in the sugars could be detected, and the method offers a possible means for locating their positions.[120]

Of the monosaccharide derivatives hyperoside (3-*O*-β-D-galactopyranosylquercitin) has been isolated for the first time from the *Aster* species,[121] and 7,4-dibenzylquercitin was condensed with the acetobromo-derivatives of D-glucose, D-galactose, and L-rhamnose, and removal of the blocking groups from the products afforded the flavonoid glycosides isoquercitrin, hyperoside, and quercitrin, respectively.[122] The glycolipids extracted from wheat flour were found to consist of 1-*O*-(6-*O*-acetyl-β-D-galactopyranosyl)-2,3-di-*O*-acyl-D-glyceritols and phytosteryl 6-*O*-acetyl-β-D-glucopyranosides.[123] *O*-β-D-Glucopyranosylmaltol (56), a new glycoside isolated from



(56) X=O
(57) X=NH



(58)

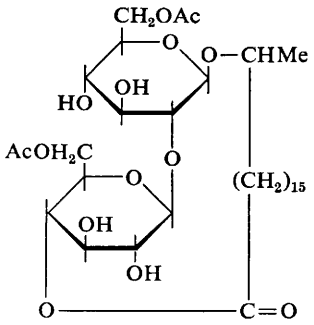
the leaves of *Evodiopanax innovans*, was synthesised by a Koenigs–Knorr reaction using the potassio derivative of maltol. With ammonia in aqueous solution (56) was converted to (57).[124]

A further study of ranunculin, the glucoside precursor of the vesicant principle of many *Ranunculus* species, established its full structure as (58)

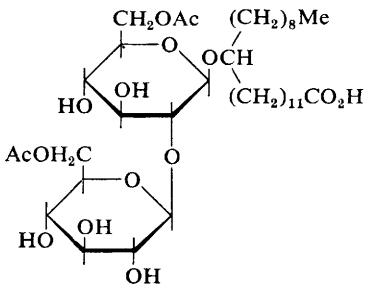
[118] R. D. Poretz and I. J. Goldstein, *Arch. Biochem. Biophys.*, 1968, **125**, 1034.
[119] H.-W. Voigtländer and G. Balsam, *Arch. Biochem.*, 1968, **301**, 208.
[120] I. A. Pearl and S. F. Darling, *Phytochemistry*, 1968, **7**, 831.
[121] N. R. Farnsworth, H. Wagner, L. Hörhammer, and H.-P. Hörhammer, *J. Pharm. Sci.*, 1968, **57**, 1059.
[122] L. Hörhammer, H. Wagner, H.-G. Arndt, R. Dirscherl, and L. Farkas, *Chem. Ber.*, 1968, **101**, 450.
[123] D. V. Myhre, *Canad. J. Chem.*, 1968, **46**, 3071.
[124] M. Yasue, N. Kawamura, and K. Ishibashi, *J. Pharm. Soc. Japan*, 1968, **88**, 390.

which has apparent relationship to a pyranosyl-furanoid disaccharide.[125] Neohesperidose (2-*O*-α-L-rhamnopyranosyl-D-glucopyranose) was crystallised for the first time,[126] and the disaccharide glycosides, sophorosyl quercitin, and kampferol,[127] and 6-*O*-α-L-rhamnopyranosyl-D-glucopyranosyl quercetin were synthesised.[128] Two mannobioses isolated from the partial hydrolysate of ovomucoid and orosomucoid were shown to be linked 1 → 3 and 1 → 6.[129]

A series of sophorosides of 17-L-hydroxyoctadecanoic acid are produced in the glycolipids of a yeast of the genus *Torulopsis*. Compound (59) was found to be the main product (41%) and the corresponding acid was found in 31% yield. The lactone ring of (59) stabilised the 2,3-diol to oxidation by



(59)



(60)

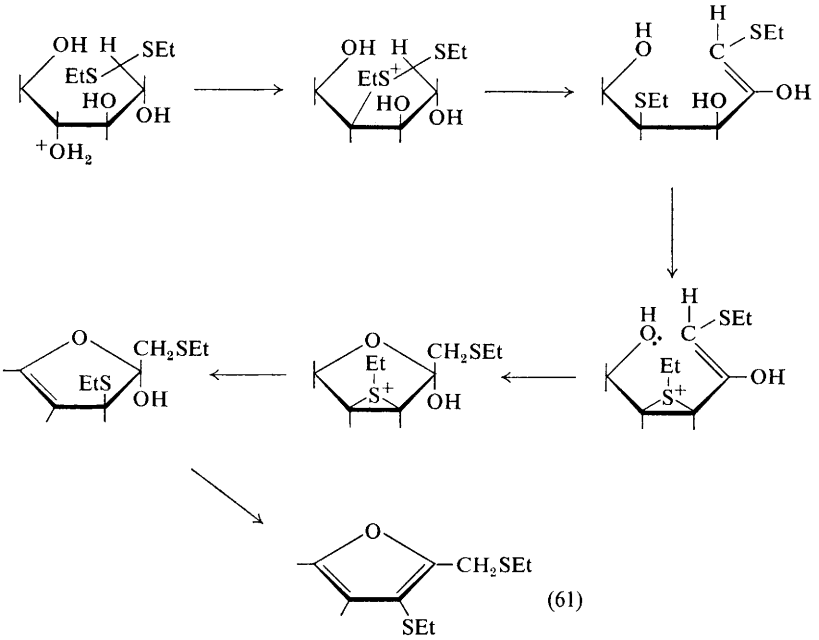
periodate, and also in the paper a wide range of sophorose derivatives and their reactions were reported.[130] A related glycoside (60) was found to be the principal component of the extracellular glycolipids of *Candida bogoriensis*.[131]

Centose isolated from the fractionation of honey has been shown to be *O*-α-D-glucopyranosyl-(1 → 4)-*O*-[α-D-glucopyranosyl-(1 → 2)]-D-glucose,[132] and the trisaccharides of plants of the *Amaryllidaceae* family have been examined by t.l.c. and several fructosyl-sucroses were separated and identified.[133]

[125] M. H. Benn and L. J. Yelland, *Canad. J. Chem.*, 1968, **46**, 729.
[126] B. H. Koeppen, *Tetrahedron Letters*, 1968, 2393.
[127] H. Wagner, L. Hörhammer, R. Dirscherl, L. Farkas, and M. Nógrádi, *Chem. Ber.*, 1968, **101**, 1186.
[128] L. Hörhammer, H. Wagner, H.-G. Arndt, G. Hitzler, and L. Farkas, *Chem. Ber.*, 1968, **101**, 1183.
[129] B. Fournet, G. Takerkart, J. Brohon, and J. Montreuil, *Bull. Soc. Chim. Biol. France*, 1958, **50**, 1351.
[130] A. P. Tulloch, A. Hill, and J. F. T. Spencer, *Canad. J. Chem.*, 1968, **46**, 3337.
[131] A. P. Tulloch, J. F. T. Spencer, and M. H. Deinema, *Canad. J. Chem.*, 1968, **46**, 345.
[132] I. R. Siddiqui and B. Furgala, *Carbohydrate Res.*, 1968, **6**, 250.
[133] H. Hammer, *Acta Chem. Scand.*, 1968, **22**, 197.

S-Glycosides

Details of the reaction of D-xylose with ethanethiol in DMF in the presence of hydrochloric acid have been elucidated using radioactive sugar. The initial products were the furanosides (α-predominating) which anomerised and were converted to the diethyl dithioacetal at approximately the same rate; at no stage did the pyranosides exceed 6%. Eventually the main product was the acetal, but after prolonged reaction times a degradation product (61) was produced, the proposed route to which is shown in

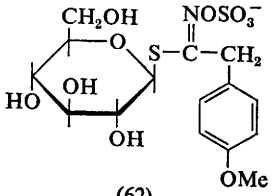


Scheme 5

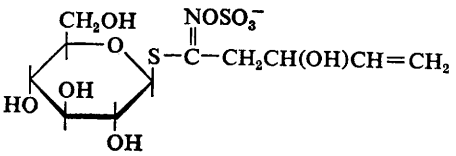
Scheme 5.[134] Reference to studies on the acid-catalysed hydrolyses of thioglycosides has been made earlier.[105]

A newly discovered naturally occurring thioglycoside (62) was isolated as its crystalline tetramethylammonium salt from the seeds of the crucifer *Aubrietia*,[135] and *epi*-progoitrin (63), the main thioglycoside of crambe seed meal (a crucifer seed product of potential interest as a feed additive), was shown to be degraded non-enzymically by iron(II) salts to give (64) or (65). This degradation was studied in detail and the structural features of thioglycosides required to give thionamides were determined.[136]

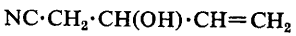
[134] R. J. Ferrier, L. R. Hatton, and W. G. Overend, *Carbohydrate Res.*, 1968, **6**, 87.
[135] R. Gmelin, A. Kjaer, and A. Schuster, *Acta Chem. Scand.*, 1968, **22**, 713.
[136] F. L. Austin, C. A. Gent, and I. A. Wolff, *J. Agric. Food Chem.*, 1968, **16**, 752.



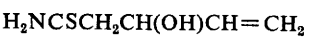
(62)



(63)



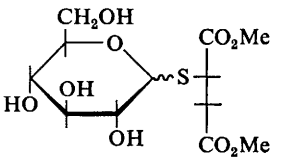
(64)



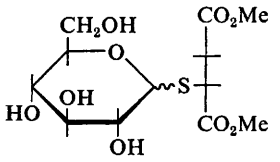
(65)

In the synthetic field a series of *S*-glycosides including hexosides, pentosides, and a glucofuranuronosyl derivative of 6-mercaptopurine was synthesised, (i) by treating the mercaptopurine with acylglycosyl halides and (ii) by treating 6-halogenopurines with 1-thioglucose derivatives. The products were readily oxidisable to sulphones and were readily hydrolysed by acids or suitable enzymes.[137] In similar fashion, glucosylation of 2-mercaptopyrimidine with acetobromoglucose gave the thioglycoside (together, though, with the *N*-substituted isomer) which could also be prepared from penta-acetyl-1-thio-D-glucose and 2-chloropyrimidine. Sulphur-to-nitrogen migration occurred on heating the product.[138] In like manner, the reaction of acetobromoglucose with imidazole-, oxazole-, and thiazole-2-thiones was found to give, under S_N2 conditions, mainly 2-thioglucosides, whereas under unimolecular conditions these were formed only if the heterocyclic ring contained a large substituent at C-4; in the absence of such groupings glucosylamines were formed.[139] In conjunction with this work the n.m.r. spectra of several hetero-aromatic α-D-thioglucosides have been reported; they showed that the sugar ring adopted the *C*1 conformation.[140]

A more novel synthesis of thioglycosides involved the addition of 1-thio-D-glucopyranose or its 2,3,4,6-tetra-acetate to the double bond of dimethyl maleate or fumarate to give mixtures of compounds (66) and (67) or their



(66)



(67)

[137] I. Goodman, L. Salce, and G. H. Hitchings, *J. Medicin. Chem.*, 1968, **11**, 516.
[138] G. Wagner and F. Suess, *Die Pharmazie*, 1968, **23**, 8.
[139] P. Nuhn and G. Wagner, *Arch. Pharm.*, 1968, **301**, 186.
[140] P. Nuhn, W. Bley, and G. Wagner, *Arch. Pharm.*, 1967, **300**, 926.

acetates. Similarly, 1-thioglucose gave a water-soluble high molecular-weight adduct with fumaric acid, butane-1,3-diol polyester showing strong surface-active properties.[141]

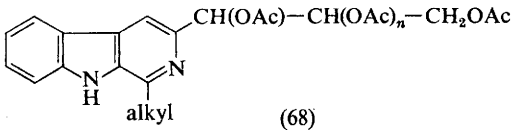
A few simple derivatives of ethyl 1-thio-β-D-glucopyranoside have been reported; these are the 4,6-*O*-benzylidene derivative and its diacetate and dibenzoate, and ethyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside.[142]

C-Glycosides

The isolation of a series of *C*-D-glucosyl and *C*-D-xylosyl flavones from citrus fruits has been described (including new compounds). U.v. and paper chromatographic and electrophoretic properties of the compounds were compared, and from the chemical shifts of the acetyl protons in the n.m.r. spectra of the derived acetates, the positions of glycosylation on the flavones could be determined.[143] Other workers have extracted a series of *C*-glycosides from *Passiflora incarnata* including saponarin, vitexin, orientin, and *homo*-orientin.[144]

In synthetic work *C*-glycosylation of the flavone diosmetin or its 3′-benzyl ether using acetobromoglucose gave the 6-substituted β-compound found to be identical with a *C*-glycoside of lemon rinds.[145]

The synthesis of a variety of acyclic compounds related to *C*-glycosides of β-carboline and having the general structure (68) have been reported



(68)

(D-*galacto* and L-*arabino* configuration).[146] Mass spectra of *C*-glycosides have been reported (see p. 200).

[141] F. Micheel and W. Kriesten, *Chem. Ber.*, 1968, **101**, 3724.
[142] A. F. Bochkov and A. C. Jain, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 179.
[143] B. Gentili and R. M. Horowitz, *J. Org. Chem.*, 1968, **33**, 1571.
[144] H. Schilcher, *Z. Naturforsch.*, 1968, **23B**, 1393.
[145] J. Chopin, A. Durix, M.-L. Bouillant, and J. Wallach, *Compt. rend.*, 1968, **267C**, 1722.
[146] Yu. A. Zhdanov, V. I. Kornilov, and G. N. Dorofeenko, *Doklady Akad. Nauk S.S.S.R.*, 1968, **178**, 1007.

4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—Very little work has been reported specifically on methyl ethers, but as always, these derivatives were employed by many workers for analytical, gas chromatographic, and mass spectrometric purposes. Mass spectrometry can be used for the determination of the number and position of methyl groups on carbohydrates (see p. 199).

The relative rates of methylation (using dimethyl sulphate in strong aqueous sodium hydroxide solution) of the hydroxy-groups of dextran (1,6-α-glucosan), pustulan (1,6-β-glucosan), and various monomeric model compounds have revealed that the hydroxyl at C-2 always methylated most readily, and that at C-3 least readily. The relative reactivities were similar in the polymers and in the monomers, indicating that phenomena associated with the monomeric species are mainly responsible for determining the ease of substitution of the hydroxy-groups. Substituents at C-6 apparently increased the relative reactivities of position 4, and the order of reactivity was not greatly altered by inverting the pyranoid ring conformations since the general pattern was retained in going from methyl glucopyranoside to 1,6-anhydro-D-glucose. In a competitive experiment dextran and methyl 6-*O*-(tetrahydropyranyl)-α-D-glucopyranoside methylated at approximately equal rates.[147]

Japanese workers have used a micro-methylation procedure (0·5–1·0 mg.) employing methyl iodide, sodium hydride, and DMF for preparing methyl ethers of a wide variety of glucuronides which were examined by g.l.c.[148]

By standard techniques the hitherto unknown 2,5,6- and 3,5,6-trimethyl ethers of D-galactofuranose were synthesised.[149] 3,5-Di-*O*-methyl-D-mannose has also been prepared by a method involving selective 2-sulphonylation of methyl 6-*O*-triphenylmethyl-α-D-mannofuranoside.[150] The methanolysis of 2,3,4,5-tetra-*O*-methyl-D-glucose afforded the β-septanoside.[60]

The mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose has been studied,[21-23] and is described in Chapter 6.

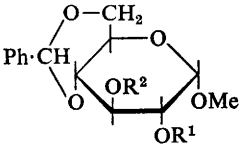
[147] B. Norrman, *Acta Chem. Scand.*, 1968, **22**, 1623.
[148] T. Imanari and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1677.
[149] I. R. Siddiqui and B. Urbas, *Carbohydrate Res.*, 1968, **7**, 80.
[150] I. R. Siddiqui and V. L. N. Murty, *Carbohydrate Res.*, 1968, **8**, 477.

Two papers have reported the partial methylation with diazomethane of nucleosides. Adenosine and cytidine gave the 2′- and 3′-ethers in the ratio 3 : 1,[151] while other workers obtained from adenosine the 2′-, 3′-, 5′-, and 2′,3′-derivatives in the ratios 38 : 11 : 1·5 : 3·5. (These figures are also the % yields obtained.[151a])

Substituted Alkyl Ethers.—Methyl 3-*O*-ethyl-α-D-glucopyranoside has been prepared from the 4,6-*O*-benzylidene 2-*O*-toluene-*p*-sulphonyl derivative and from 3-*O*-ethyl-1,2:5,6-di-*O*-isopropylidene-D-glucose.[152] The benzylation of a variety of carbohydrates in DMSO has been carried out with several bases, the most efficient of which were sodium hydride, sodium or potassium hydroxide, and sodamide.[153] Specifically, 6-*O*-benzyl-D-galactose and its dimethyl acetal have been synthesised from the readily available 6-*O*-benzyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose.[154] Also, 2,3,4-tri-*O*-benzyl-α-D-xylopyranose and 2,3,4-tri-*O*-benzyl-β-D-ribopyranose have been described.[155]

In the nucleoside series benzylation of uridine with sodium hydride–benzyl bromide in DMSO or DMF gave 3-*N*-benzyluridine and the 2′-*O*,3-*N*-dibenzyl derivative; the authentic 2′-*O*,3-*N*- and 3′-*O*,3-*N*-compounds being prepared by benzylation of the 3′,5′- and 2′,5′-ditrityl ethers respectively.[156] Alternatively, benzylation of 4-methylthiouridine or of cytidine gave specifically the 2′-*O*-benzyl ethers, both of which were converted to 2′-*O*-benzyluridine, an important intermediate in oligonucleotide syntheses.[157]

Full details have appeared on the removal of the allyl ether protecting group (using mercuric salts in aqueous media) in the presence of acid-labile substituents (*cf.* Vol. 1, p. 47); *e.g.*, 4,6-*O*-benzylidene-D-galactose was prepared from the prop-1-enyl glycoside. Preferential rearrangement of the 2-*O*-allyl group of methyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (69) gave the 3-*O*-allyl-2-*O*-prop-1′-enyl derivative (70) a useful precursor of 2-*O*-substituted glucoses. Removal of the vinyl ether



	R^1	R^2
(69)	$CH_2{\cdot}CH{:}CH_2$	$CH_2{\cdot}CH{:}CH_2$
(70)	$CH{:}CH{\cdot}CH_3$	$CH_2{\cdot}CH{:}CH_2$
(71)	$CH_2{\cdot}CH{:}CH_2$	$CH{:}CH{\cdot}CH_3$

[151] D. M. G. Martin, C. B. Reese, and G. F. Stephenson, *Biochemistry*, 1968, **7**, 1406.
[151a] J. B. Gin and C. A. Dekker, *Biochemistry*, 1968, **7**, 1413.
[152] J. T. Marvel, S. K. Ken, J. W. Berry, and A. J. Deutschman jun., *Carbohydrate Res.*, 1968, **8**, 148.
[153] T. Iwashige and H. Saeki, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 1803.
[154] E. F. L. J. Anet, *Carbohydrate Res.*, 1968, **7**, 84.
[155] S. Tejima, R. K. Ness, R. L. Kaufman, and H. G. Fletcher jun., *Carbohydrate Res.*, 1968, **7**, 485.
[156] N. Imura, T. Tsuruo, and T. Ukita, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1105.
[157] K. Kikugawa, F. Sato, T. Tsuruo, N. Imura, and T. Ukita, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1110.

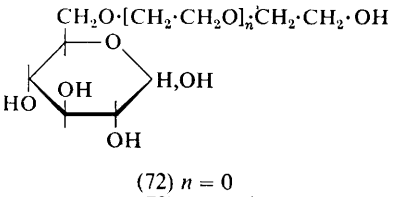
from (70), rearrangement of the 3-*O*-allyl group, and subsequent allylation gave the 2-*O*-allyl-3-*O*-prop-1′-enyl derivative (71), correspondingly a possible compound from which 3-*O*-substituted compounds could be prepared. Rearrangement of an allyl group in the presence of the benzamido-group occurred with hydrolysis of the latter.[158] Preliminary studies by the same workers on crotyl ethers of carbohydrates suggested that their removal by potassium t-butoxide in DMSO may make them useful protecting groups.

Allylcellulose (partially substituted) was shown to have the ether groups distributed between the hydroxy-groups of C-2, C-3, and C-6 in the ratio 0·7 : 0·2 : 1·0. This was determined by gass chromatographic examination of the TMS ethers of the polymer hydrolysate; for the work 2-, 3-, and 6-*O*-allyl-D-glucose were synthesised by essentially standard routes.[159]

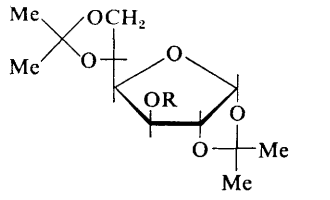
The 2,3-di-*O*-vinyl ether of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside was prepared using acetylene in dioxan in the presence of potassium hydroxide. In aqueous dioxan, monovinylation occurred and the 3-*O*-vinyl derivative was obtained, but the 2-*O*-vinyl ether (identified by n.m.r. spectroscopy of the crude reaction mixture) rearranged to the 2,3-*O*-ethylidene acetal during chromatography on alumina. Reduction of the di- or mono-ethers gave the expected ethylated derivatives.[160] In related fashion various unsaturated ethers of D-glucose derivatives were prepared and converted by hydrogenation into the corresponding saturated alkyl ethers. The following such derivatives were obtained: 1,2-*O*-chloroethylidene-3,5,6-tri-*O*-vinyl, 1,2,3-tri-*O*-allyl-4,6-*O*-ethylidene, 3,5,6-tri-*O*-allyl-1,2-*O*-trichloroethylidene, 4,6-*O*-ethylidene-1,2,3-tri-*O*-crotyl, and 3,5,6-tri-*O*-crotyl-1,2-*O*-trichloroethylidene. Propargyl ethers (of mannitol as well as of polysaccharides) on iodination afford ethers useful in biomedical work; radio-iodine can be incorporated into living systems in this form.[161] Several unsaturated ethers (vinyl, allyl and crotyl) of D-glucose derivatives have been prepared and hydrogenated to the corresponding saturated alkyl ethers.[161a]

Work by Hook and Lindberg on the hydrolysis of the hydroxyethyl ethers of the methyl D-glucopyranosides has already been mentioned [108] as has the intramolecular glycosidation of 2-*O*-hydroxyethyl-D-glucose.[62] In other work the synthesis of 6-*O*-(2-hydroxyethyl)-D-glucose (72) and its ethylene oxide adducts (73) has been described. The homologues were separated by chromatography on anion-exchange resins and characterised by paper chromatography, g.l.c., and mass spectrometry of the TMS derivatives.[162]

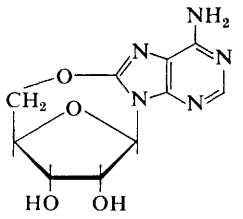
[158] R. Gigg and C. D. Warren, *J. Chem. Soc.* (*C*), 1968, 1903.
[159] D. E. Hoiness, C. P. Wade, and S. P. Rowland, *Canad. J. Chem.*, 1968, **46**, 667.
[160] J. T. Marvel, S. K. Sen, F. T. Uenaka, J. W. Berry, and A. J. Deutschman jun., *Carbohydrate Res.*, 1968, **6**, 18.
[161] M. Tubis, K. Parsons, J. S. Endow, S. S. Rawalay, and P. H. Crandall, *J. Nuclear Medicin.*, 1967, **8**, 551.
[161a] B. I. Mikhantjev, V. L. Lapenko, and V. E. Sopina, *Zhur. obschei Khim.*, 1968, **38**, 2616.
[162] O. Ramnäs and O. Samuelson, *Carbohydrate Res.*, 1968, **6**, 355.



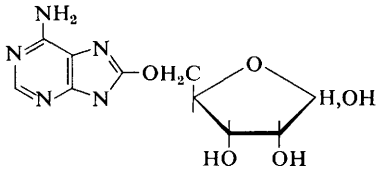
(72) $n = 0$
(73) $n = 1$–4



(74) R = $CH_2 \cdot CH(OH) \cdot [CH_2]_5 \cdot CH_3$



(75)



(76)

Various long-chain alkylated derivatives of D-glucose were synthesised by utilising the base-catalysed ring-opening reaction undergone by epoxides. The terminal epoxides of 1,2-epoxyoctane and 1,2-epoxyoctadecane were used to etherify 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [*e.g.* to give (74)], and subsequent removal of the acetal rings gave 3-*O*-substituted glucoses. Similar treatment of 1,2-*O*-isopropylidene-α-D-glucofuranose and of methyl α-D-glucopyranoside gave mono-substituted products.[163]

Acidic hydrolysis of 8,5′-anhydro-8-hydroxyadenosine (75) afforded not just 8-hydroxyadenosine, but also the 5′-ether (76).[164]

Silyl Ethers.—Trimethylsilyl ethers continue to be used extensively in g.l.c. and mass spectrometry studies of carbohydrates, and it has now been demonstrated that they are sufficiently stable to be subjected to t.l.c. With dry benzene as eluting solvent the isomeric penta-*O*-trimethylsilyl-D-glucopyranoses (and the methyl glucopyranoside tetraethers) have been resolved satisfactorily.[165]

By a combination of g.l.c. and mass spectrometry the five components detected in pertrimethylsilylfructose were shown to be the two pyranosides, the two furanosides, and the acyclic keto form.[166]

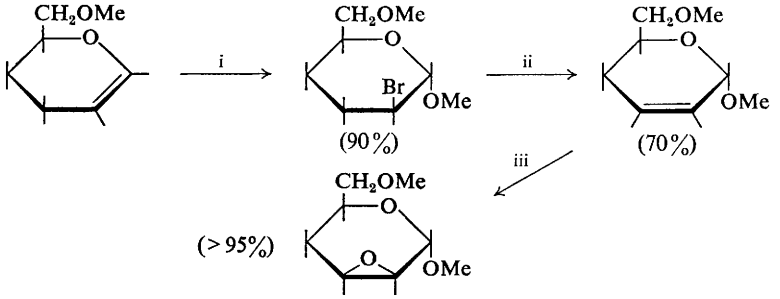
Complex polymeric products were obtained on treating sucrose (and independently tetrahydro-2-hydroxymethylpyran as a model compound) with dichlorodimethyl- and dichlorodiphenyl-silane. The effects on the products of water, methanol, and ammonia were investigated.[167]

[163] F. Micheel and P. Schiller, *Chem. Ber.*, 1968, **101**, 3721.
[164] M. Ikehara and M. Kaneko, *J. Amer. Chem. Soc.*, 1968, **80**, 497.
[165] J. Lehrfeld, *J. Chromatog.*, 1968, **32**, 685.
[166] H. C. Curtius, M. Müller, and J. A. Völlmin, *J. Chromatog.*, 1968, **37**, 216.
[167] S. A. Barker and M. R. Harnden, *J. Chem. Soc.* (*C*), 1968, 644.

Selective 6-phosphorylation of a galactoside has been effected by use of the 2,3,4-tri-*O*-trimethylsilyl ether obtained by partial hydrolysis of the tetraether (see p. 54).

Intramolecular Ethers (Anhydro-sugars)

Epoxides.—Several papers have appeared on the synthesis and reactions of glycoside epoxides synthesised from racemic dihydropyrans. Thus, methyl 2,3-anhydro-4-deoxy-6-*O*-methyl-α-DL-*lyxo*-hexopyranoside was prepared as shown in Scheme 6.[168] Reaction of *cis,trans*-3,4-epoxy-2-methoxytetrahydropyran with methanol in the presence of an acid catalyst gave methyl



Reagents: i, NH_3, MeOH, Br_2; ii, NaOMe; iii, $Cl \cdot C_6H_4 \cdot CO_3H$

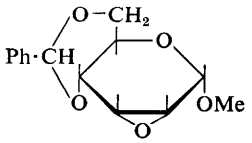
Scheme 6

4-deoxy-3-*O*-methyl-α,β-DL-*threo*-pentopyranoside in good yield showing that such epoxides open preferentially by attack at C-3. A similar finding was obtained during ring-opening with lithium aluminium hydride.[169]

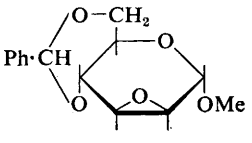
Double resonance experiments have shown that in a series of 3,4-epoxy-2-alkoxytetrahydropyrans the epoxide-ring proton nearer the anomeric centre resonates at higher fields than the other. This was in contrast with conclusions drawn by other authors on the closely related methyl 2,3-anhydro-4,6-*O*-benzylidene-hexopyranosides.[170] A useful generalisation was re-emphasised in this paper: for epoxides on six-membered rings, epoxide protons show almost zero coupling with their neighbours when they have the *trans* relationship. For *cis* related protons $J = 2{\cdot}5$–$4{\cdot}5$ Hz.

An apparent anomaly in the literature relating to the products formed on treatment of (77) with methyl-lithium has been shown to arise from variable purity of the reagent. Evidence was produced which showed that halide-free methyl-lithium gave a branched-chain methyl glycoside which reacted further to give a 2-*C*-methylallal derivative. Alternatively, when

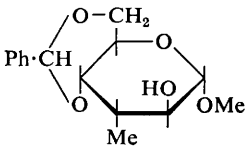
[168] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 2283.
[169] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 707, 1592.
[170] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 1481.



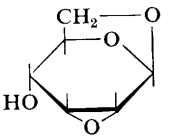
(77)



(78)



(79)



(80)

halide was present in the reagent it reacted preferentially to give a 2-deoxy-2-halogenoaltroside derivative which with the metal alkyl gave 4,6-*O*-benzylidene-D-allal. The corresponding mannoside (78) with methyl–lithium–lithium iodide did not give an iodo-intermediate but rather (79).[171]

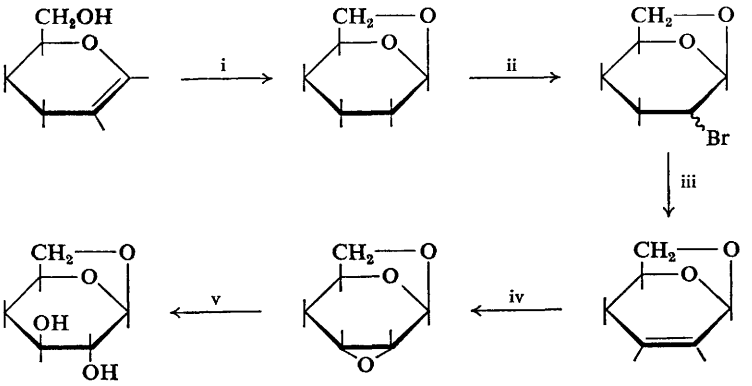
Ring-opening of compound (80) with alkali gave the product with the *gluco* structure in keeping with the expectation that the isomer with diaxial hydroxy-groups would predominate.[172] Reductions of methyl 3,4-anhydro-6-deoxy-α-D-galactopyranoside have been examined (p. 124), and the reactions undergone by epoxides on heating with sodium cobalt tetracarbonyl in the presence of carbon monoxide in methanol and with aluminium triethyl–hydrogen cyanide are discussed on pp. 129 and 106 respectively.

Other Anhydrides.—The equilibria established in acid media between aldohexoses and their 1,6-anhydrides, and between heptuloses and the corresponding 2,7-anhydrides have been determined by g.l.c. of the derived acetates and TMS ethers, and the results were found to agree closely with those expected on the basis of calculations of intramolecular interaction energies. This new information represents the best available on a topic which has received appreciable attention over several years. A full discussion was provided and consideration was given to the existence of anhydrofuranoses, and of 1,7-anhydrides as well as 1,6-anhydrides in the case of aldoheptoses, three of which were investigated.[173] 1,6-Anhydro-β-D-glucopyranose was the major product on heating methyl α-D-glucopyranoside in DMSO at 160° for 25 hr., but the 1,6-anhydrofuranose was also obtained in small amounts.[174]

The reactions of 1,6-anhydro-D-glucose with dibromo- and dichloromethyl methyl ether are outlined on p. 76.

[171] M. Sharma and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 757.
[172] E. Zissis, *J. Org. Chem.*, 1968, **33**, 2844.
[173] S. J. Angyal and K. Dawes, *Austral. J. Chem.*, 1968, **21**, 2747.
[174] M. H. Fischer, *Carbohydrate Res.*, 1968, **8**, 354.

Continuing their investigation into the synthesis of carbohydrate derivatives from dihydropyrans, Sweet and Brown have prepared 1,6-anhydro-4-deoxy-β-DL-*xylo*-hexopyranose as shown on Scheme 7.[175] In the epoxide



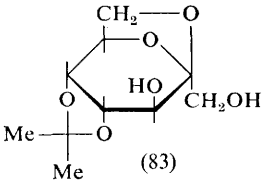
Reagents: i, H^+; ii, Br_2, CCl_4, Na_2CO_3; iii, KOH, EtOH; iv, $Cl \cdot C_6H_4 \cdot CO_3H$; v, KOH

Scheme 7

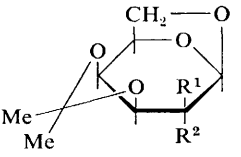
intermediate H-1 was found to be coupled to all the other protons except H-4(*exo*) (*i.e.* coupling over 4 and 5 bonds was observed), and it was concluded that long-range coupling is favoured by the arrangement (81) as well as by (82).

Oxidation of (83) with DMSO–acetic anhydride followed by reduction with sodium borohydride and partial acid hydrolysis gave 2,7-anhydro-β-D-*allo*- and *altro*-heptulopyranose (the former being a new compound, the latter the well known sedoheptulosan). From 3-*O*-toluene-*p*-sulphonyl-sedoheptulosan the 3,4-epoxide was prepared.[172] In related fashion,

(81) (82)



(83)



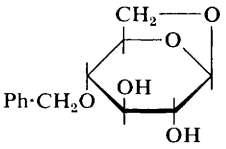
	R^1	R^2
(84)	OH	H
(85)	H	OH
(86)	OH	OH

1,6-anhydro-3,4-*O*-isopropylidene-β-D-talopyranose (84) has been synthesised by oxidation of the *galacto*-epimer (85) with DMSO–acetic anhydride. This gave the *gem*-diol (86) which was reduced with sodium

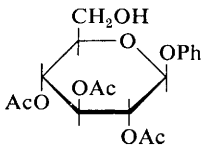
[175] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 2289.

borohydride. 1,6-Anhydro-2-*O*-methyl-β-D-talose was prepared from the product.[176]

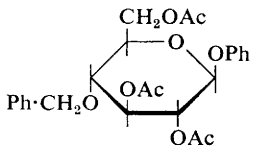
A synthesis of 1,6-anhydro-4-*O*-benzyl-β-D-glucopyranose (87) from phenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (88) has been reported. Benzylation of (88) with benzyl bromide in DMF in the presence of



(87)



(88)



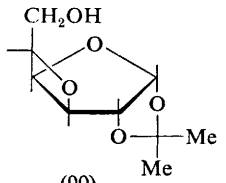
(89)

silver oxide occurred after migration of the C-4 acetyl group and the required phenyl 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-D-glucopyranoside (89) was obtained.[176a]

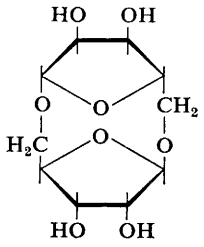
6-*O*-Methanesulphonyl- and -toluene-*p*-sulphonyl-trehalose have been prepared by preferential esterification of one primary hydroxy-group of the disaccharide. LAH reduction of the acetylated derivative of the latter ester gave not the 6-deoxy-derivative but instead 3,6-monoanhydrotrehalose.[177]

Treatment of 1,2-*O*-isopropylidene-3,5-di-*O*-toluene-*p*-sulphonyl-D-xylofuranose with methanolic hydrogen chloride gave the 2,5-anhydro-derivative (see p. 73). Improvements in the preparation of (90) have been described, and on treatment with sodium azide followed by reduction 5-amino-5-deoxy-1,2-*O*-isopropylidene-D-glucofuranose was obtained (see p. 82).

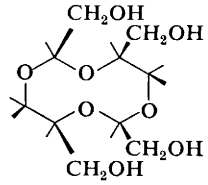
The known dianhydride of D-ribose has been shown to be the β-ribofuranose dimer (91) by chemical means following its partial hydrolysis to



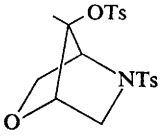
(90)



(91)

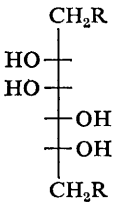


(92)

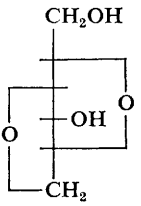


(93)

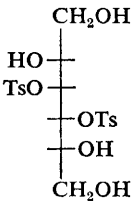
[176] N. A. Hughes, *Carbohydrate Res.*, 1968, **7**, 474.
[176a] P. A. Seib, *Carbohydrate Res.*, 1968, **8**, 101.
[177] E. Guilloux and F. Percheron, *Compt. rend.*, 1968, **266C**, 153.



R = Cl or Ms
(94)



(95)



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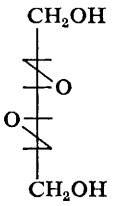


(97)

5-*O*-*β*-D-ribofuranosyl-D-ribofuranose. The *β*-configurations were confirmed by n.m.r. spectroscopy which also allowed the determination of the overall conformation of the molecule. Periodate oxidation followed by reduction of the derived aldehydic functions gave (92) the conformational properties of which were also examined by n.m.r. and discussed in full.[178] 2,5-Anhydro-D-ribose derivatives have been converted into 3-oxa-6-azabicyclo[2,2,1]heptanes, *e.g.* (93).[179]

Reaction of 1,6-di-*O*-methanesulphonyl- or 1,6-dichloro-1,6-dideoxy-D-mannitol (94) with sodium methoxide in methanol gave a product which was established as 2,5:3,6-dianhydro-D-glucitol (95), the configuration of which shows that an inversion has occurred at C-2 and consequently that at least one terminal epoxide had been formed as an intermediate.[83]

The previously reported reaction of 3,4-di-*O*-toluene-*p*-sulphonyl-D-mannitol (96) with methoxide has been reinvestigated and was found to give 2,3:4,5-dianhydro-D-iditol (97).[180]

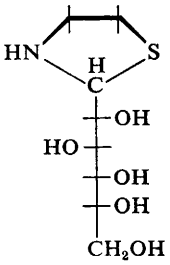
[178] J. F. Stoddart and W. A. Szarek, *Canad. J. Chem.*, 1968, **46**, 3061.
[179] J. Cleophax, S. D. Gero, and A. M. Sepulchre, *Carbohydrate Res.*, 1968, **7**, 505.
[180] R. S. Tipson and A. Cohen, *Carbohydrate Res.*, 1968, **7**, 232.

5
Acetals

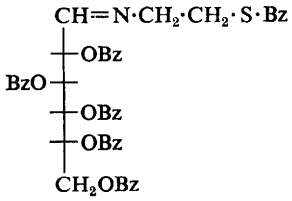
Acetals Derived from Carbohydrate Carbonyl Groups

For the first time acetals have been detected in the methanolysis products of free sugars; they were shown, however, to be formed in reactions competitive with the main glycosylation processes, and were thus considered not to be important intermediates. It was concluded, alternatively, that the hemiacetals most probably were intermediates formed *en route* to the furanosides.[54, 55]

The dimethyl acetal of 6-*O*-benzyl-D-galactose has been reported.[154] A large number of derivatives of (98), which can be looked on as an acetal analogue, were described including esters and acetals. Benzoylation in aqueous alkali gave a ring-opened product (99). Compounds of the 4-amino-4-deoxy-D-*galacto*-series were obtained by nucleophilic displacements of 4-sulphonyloxy-groups from products obtained by way of 2,3:5,6-diacetals.[181]



(98)



(99)

Acetals Derived from Carbohydrate Hydroxy-groups

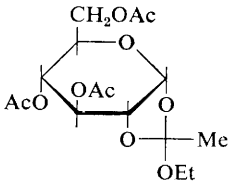
The potentially useful observation has been made that 90% aqueous trifluoroacetic acid at room temperature will cleave isopropylidene and benzylidene acetals without influencing halogeno-, benzoyl, sulphonyl, amino- or azido-groups on the same molecules.[182]

[181] T. Takatori and T. Taguchi, *J. Pharm. Soc.* (*Japan*), 1968, **88**, 527.
[182] J. E. Christensen and L. Goodman, *Carbohydrate Res.*, 1968, **7**, 510.

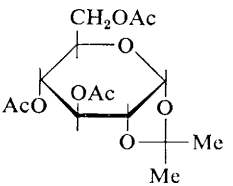
From Diols on Acyclic Carbohydrates.—2,3-*O*-Butylidene-D-glucitol has been shown to be formed from D-glucitol and n-butyraldehyde in aqueous acid under kinetic control. Study of the reaction by g.l.c. of the TMS derivatives showed that as equilibrium was approached this product isomerised to the 2,4-acetal. Comparative hydrolysis studies showed that the 2,3-compound hydrolysed much more readily than its isomer.[183] Similarly, acetaldehyde and benzaldehyde gave initially 2,3-products which slowly isomerised.[184] These isomerisations also proceeded in anhydrous media.

Acid-catalysed condensation of bromoacetaldehyde diethyl acetal with D-mannitol afforded two crystalline products, both of which were shown by chemical methods to possess 1,2:5,6-di-*O*-bromoethylidene structures. By consideration of the chemical shifts of the acetal protons the diastereoisomers were shown to have the substituents on the dioxolan rings in the *cis,cis*- and *cis,trans*-relationships, respectively.[185] Benzylidenation reactions of pentonic acid derivatives are referred to on p. 156.

From Diols on Cyclic Carbohydrates.—(a) *Free Sugars.* A new synthesis of 1,2-*O*-alkylidenealdoses, from treatment of 1,2-orthoesters with ketones in the presence of toluenesulphonic acid, *e.g.* (100) → (101), has been

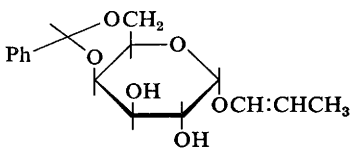


(100)

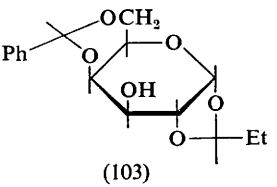


(101)

described. Completely dry conditions were necessary and were assured by the addition of trimethyl orthoformate to the ketone, prior to the introduction of the carbohydrate.[186] In similar fashion, acid-catalysed reaction of prop-1′-enyl glycosides with unsubstituted hydroxy-groups at C-2 gave 1,2-*O*-propylidene acetals for example (102) → (103).[158]



(102)

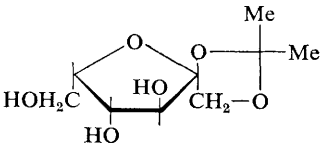


(103)

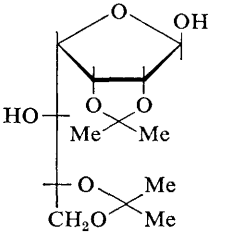
[183] T. G. Bonner, E. J. Bourne, P. J. V. Cleare, and D. Lewis, *J. Chem. Soc.* (*B*), 1968, 822.
[184] T. G. Bonner, E. J. Bourne, P. J. V. Cleare, and D. Lewis, *J. Chem. Soc.* (*B*), 1968, 827.
[185] H. B. Sinclair, *J. Org. Chem.*, 1968, **33**, 3714.
[186] R. U. Lemieux and D. H. Detert, *Canad. J. Chem.*, 1968, **46**, 1039.

Conditions have been given for the resin-catalysed partial hydrolysis of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose and for the isolation of the 1,2-acetal in 89% yield.[187]

Following their work on the acetonation of L-sorbose (Vol. 1, p. 48), Maeda and his group have now studied the acetalation of this ketose with acetaldehyde and benzaldehyde and found that the results followed those obtained with acetone.[188] The Indian group interested in these same reactions have now reported on the two monoacetals and three diacetals isolated from the products formed using acetone and cupric sulphate as catalyst. The 2,3:4,6- and 1,2:4,6-diacetals were identified, and one of the monoacetals was believed to be the 1,2-α-furanose compound (104), since it was obtained by partial hydrolysis of the 1,2:4,6-diketal.[189] Such an argument is, of course, open to the criticism that rearrangements could have occurred either before or after hydrolysis. The i.r. spectra of compounds of this class are referred to on p. 198.



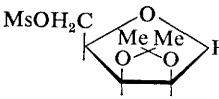
(104)



(105)

Acetonation of D-*glycero*-D-*gulo*-heptose gave, as the major product, the 2,3:6,7-di-*O*-isopropylidene compound, shown by n.m.r. and chemical studies to exist in the furanoid form (105).

A synthesis of 5-*O*-methanesulphonyl-2,3-*O*-isopropylidene-D-lyxose (106) was carried out from 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose



(106)

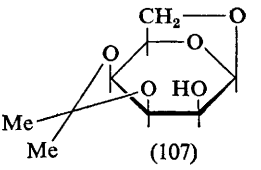
by its conversion to the benzyl α-glycoside, removal of the 5,6-acetal, periodate oxidation, and sodium borohydride reduction, followed by sulphonylation and reductive removal of the benzyl protecting groups. Hydrolysis of the intermediate benzyl 2,3-*O*-isopropylidene-α-D-lyxofuranoside offers an attractive alternative synthesis of the pentose.[190]

[187] P. M. Urquiza, D. M. Martinez, and T. S. O'Dowd, *Rev. Soc. quim. Mexico*, 1968, **12A**, 58.
[188] T. Maeda and K. Tokuyama, *Tetrahedron Letters*, 1968, 3079.
[189] J. R. Patil and J. L. Bose, *Indian J. Chem.*, 1967, **5**, 598.
[190] J. S. Brimacombe, F. Hunedy, and L. C. N. Tucker, *J. Chem. Soc.* (*C*), 1968, 1381.

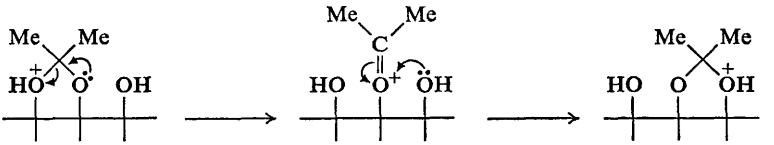
N.m.r. features of acetals of the series are referred to in Chapter 23, and the reaction of diazomethane with 1,3:4,6-di-*O*-isopropylidene-β-L-sorbofuranose is discussed on p. 142.

(b) *Glycosides.* Acetonation of methyl β-D-galactopyranoside in the presence of copper sulphate gave the 4,6-acetal in addition to the 3,4-isomer.[191] The 4,6-*O*-isopropylidene derivative of methyl β-D-glucopyranoside has been described.[192]

The solvolytic removal of benzaldehyde from a series of 2-deoxy-4,6-*O*-benzylideneglycosides with the D-*ribo*- and D-*arabino*-configurations and with a variety of groups at C-3 (OH, OMs, N_3, NH_2) as well as the 2,3-dideoxy- and 2,3-ene derivatives has been studied. For both configurations the rates were H > OH > OMs > N_3 > NH_2 and the olefin was more reactive than the 2,3-dideoxy-derivative. Compounds with axial groups at C-3 were less stable than the epimers; steric reasons were suggested.[193]



(107)

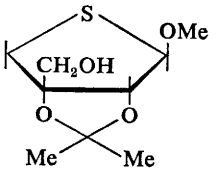


Scheme 8

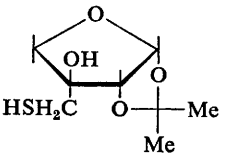
Removal of the isopropylidene group from (107) with 80% acetic acid was accompanied by the formation of the corresponding 2,3-acetal. This appears to be the first recorded example of a simple acetal rearrangement in aqueous solution and it presumably occurs as shown in Scheme 8.[194] However, another related migration occurred during the production of (107a) which was obtained by methanolysis of (108).[195] N.m.r. studies of sorboside acetals are described in Chapter 23.

From Single Alcoholic Groups.—Prior to an investigation of the positions of acetalation when starch was treated with vinyl ethers, methyl α-D-glucopyranoside was reacted with equimolar proportions of a series of alkyl vinyl ethers. In general, initial substitution was found to occur at the primary site and 6-*O*-(1′-alkoxyethyl) derivatives were isolated as the

[191] J. S. Brimacombe and O. A. Ching, *J. Chem. Soc.* (*C*), 1968, 1642.
[192] F. W. Parrish, R. C. Chalk, and L. Long jun., *J. Org. Chem.*, 1968, **33**, 3165.
[193] J. Kovář, F. Hanousek, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 630.
[194] N. A. Hughes, *Carbohydrate Res.*, 1968, **7**, 474.
[195] M. H. Halford, D. H. Ball, and L. Long jun., *Carbohydrate Res.*, 1968, **8**, 363.



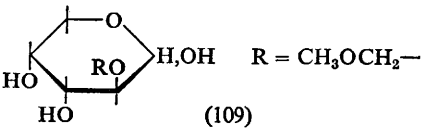
(107a)



(108)

predominant products. Subsequently, these underwent intramolecular *trans*-acetalation to give methyl 4,6-*O*-ethylidene-α-D-glucopyranosides.[196] Similarly, 3,4-dihydro-2*H*-pyran, previously shown to react unselectively with axial and equatorial hydroxy-groups of inositol derivatives, was found to react initially at position 6 of methyl α-D-glucopyranoside with good selectivity. Further reaction however caused some redistribution of the acetal; no 4,6-cyclic products were detected (although, in principle, they could still have been formed).[197]

The synthesis of 2-*O*-(methoxymethyl)-D-arabinose (109) was carried out by reaction of the sodio or potassio derivative of benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside with chloromethyl methyl ether and



(109)

removal of the protecting groups by hydrogenolysis.[198] (It is surprising that the cyclic acetal was cleaved under these conditions; conceivably sufficient acid was present to effect its selective removal.) *O*-Alkoxymethyl derivatives can also be obtained by reductive desulphurisation of thionocarbonates (see p. 54).

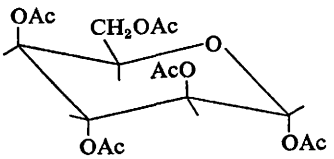
[196] M. L. Wolfrom, A. Beattie, and S. S. Bhattacharjee, *J. Org. Chem.*, 1968, **33**, 1067.
[197] M. L. Wolfrom, A. Beattie, S. S. Bhattacharjee, and G. G. Parekh, *J. Org. Chem.*, 1968, **33**, 3990.
[198] J. Csaszar and V. Bruckner, *Ann. Univ. Sci. Budapest, Sect. Chim.*, 1967, **9**, 49 (*Chem. Abs.*, 1968, **69**, 52446u).

6
Esters

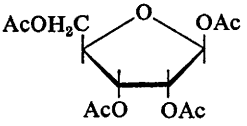
Acetates

A new synthesis of acetates involving the use of dialkythiophosphorochloridates is mentioned on p. 74.

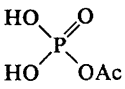
Specific acetates which have been examined are penta-*O*-acetyl-α-D-idopyranose and tetra-*O*-acetyl-β-D-ribofuranose. The former was shown by n.m.r. spectroscopy at 220 MHz to adopt the *C*1 conformation (110) in acetone and chloroform solution (see p. 194), and an improved procedure for the preparation of the latter (111) was described which affords yields of *ca.* 50%. Methyl ribofuranoside was acetylated with acetic anhydride in pyridine or with acetic anhydride and acetic acid in the presence of sulphuric acid, and in the former case the products were acetolysed with the latter reagents to give the required compound. Yields were somewhat better with the one-step procedure.[199]



(110)



(111)



(112)

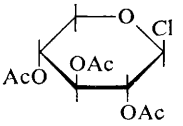
For general acetylation acetic anhydride–phosphoric acid has been recommended.[200] It was very mild in its action and was suitable for acetylation of a wide range of hydroxy-compounds including enols and primary, secondary, and tertiary alcohols. The reactive species was believed to be the monoacetic phosphoric anhydride (112), and the technique can be applied with a variety of acid anhydrides. Chlorosulphonic acid

[199] R. D. Guthrie and S. C. Smith, *Chem. and Ind.*, 1968, 547.
[200] A. J. Fatiadi, *Carbohydrate Res.*, 1968, **6**, 237.

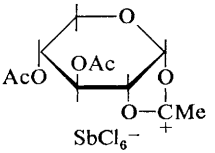
has been reported as a new catalyst for use in acetylations; for lactose and glucose the yields were higher than were obtained with conventional catalysts.[201]

Cationic resins have been described which catalysed the hydrolysis of glycosides without causing de-esterification.[202]

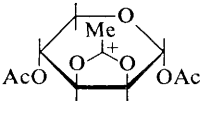
Continuing their investigations of acetoxonium ions derived from carbohydrate acetates, the Hamburg group have reported that tri-*O*-acetyl-β-D-xylopyranosyl chloride (113) reacted with antimony pentachloride in carbon tetrachloride to give a solid mixture of salts consisting of the *xylo*- (114), *lyxo*- (115), and *arabino*- (116) compounds in the ratio



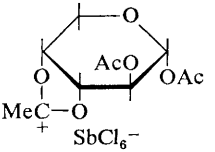
(113)



(114)



(115)

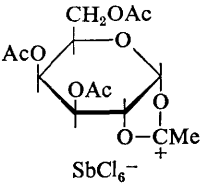


(116)

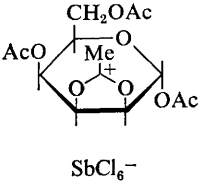
9 : 3 : 8. Acetolysis of the mixed products with acetic anhydride gave tetra-*O*-acetyl-α-D-xylopyranose (82%, 64% isolated), the β-anomer (2%), and the α-*lyxo* and α-*arabino* isomers (6 and 10%, respectively). Alternatively, hydrolysis, followed by acetylation, gave these four products in the ratio 6 : 3 : 3 : 8 and from the mixture, the α-*arabino* compound was obtained crystalline. Treatment of (113) with silver acetate and wet acetic acid gave a good yield of crystalline 1,3,4-tri-*O*-acetyl-α-D-xylopyranose.[203]

In similar fashion, 2,3,4,6-tetra-*O*-acetyl-α- or β-D-galactopyranosyl chloride (or the β-penta-acetate) with antimony pentachloride gave a mixture of the α-*galacto* and α-*talo* salts (117) and (118) in the ratio 46 : 54. On hydrolysis of the mixture followed by acetylation, 31% of penta-*O*-acetyl-α-D-talopyranose was obtained directly. Acetolysis of the salts, on the other hand, gave penta-*O*-acetyl-α-D-galactopyranose (50%). Hydrolysis of tetra-*O*-acetyl-β-D-galactopyranosyl chloride with silver acetate in wet acetic acid gave crystalline 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose in 53% yield.[204]

[201] J. Erdos and R. P. Cosio, *Ciencia*, 1967, **25**, 131 (*Chem. Abs.*, 1968, **68**, 13288d).
[202] Y. Z. Frohwein, *Nature*, 1968, **217**, 642.
[203] H. Paulsen, F. G. Espinosa, W.-P. Trautwein, and K. Heyns, *Chem. Ber.*, 1968, **101**, 179.
[204] H. Paulsen, F. G. Espinosa, and W.-P. Trautwein, *Chem. Ber.*, 1968, **101**, 186.

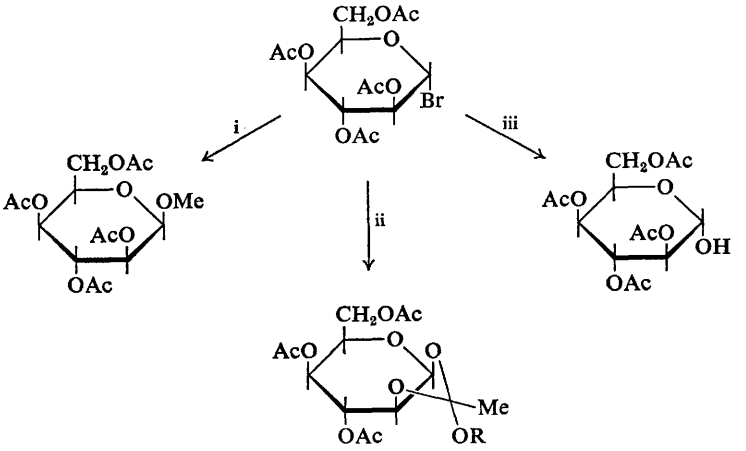


(117)



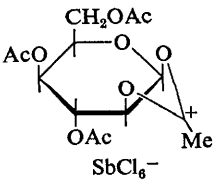
(118)

Following the development of a direct synthesis of penta-*O*-acetyl-α-D-idopyranose (Vol. 1, p. 53) the corresponding α-bromide was prepared, and its reactions were investigated (Scheme 9). The corresponding chloride with antimony pentachloride gave a mixture of the salts (119), (120), and

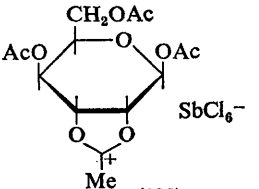


Reagents: i, MeOH–Ag_2CO_3; ii, ROH–py; iii, H_2O

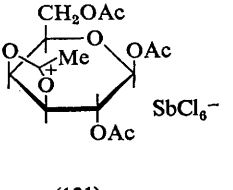
Scheme 9



(119)



(120)



(121)

(121), the hydrolysis and acetolysis of which were studied. Hydrolysis and re-acetylation offer means for obtaining penta-*O*-acetyl-β-D-galactopyranose.[205]

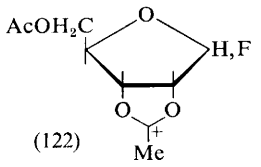
Acetoxonium ions also play important roles in the reaction of carbohydrate acetates with hydrogen fluoride. Pedersen has now shown that

[205] F. G. Espinosa, W.-P. Trautwein, and H. Paulsen, *Chem. Ber.*, 1968, **101**, 191.

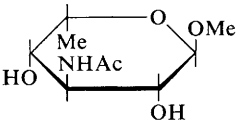
tetra-*O*-acetyl-D-arabinopyranose and -ribopyranose gave initially the β-fluorides which rearranged to give the ion (122). The corresponding pyranosyl benzoates, however, gave 1,2-benzoxonium ions (see p. 50).[206]

Acetoxonium ions also take part in acetyl migrations, such as that occurring during the detritylation of 2,3,2′,3′,4′-penta-*O*-acetyl-1,6-anhydro-6′-*O*-trityl-cellobiose which gave the 2,3,2′,3′,6′-penta-*O*-acetyl-1,6-anhydro-product.[207] A related migration occurring during a benzylation procedure has been recorded.

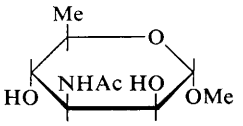
Several studies on preferential acetylations have been reported. Esterification of benzyl 4,6-*O*-benzylidene-β-D-glucopyranoside with acetic anhydride (1 mole) gave the 2- and 3-mono-acetates, in addition to some starting material and some diacetate. The mono-esters were isolated in yields of 5 and 14% respectively, and were shown to be suitable for the synthesis of 2- and 3-*O*-substituted glucose derivatives.[208] In similar fashion, partial acetylation of (123) with acetyl chloride in pyridine gave the diacetate and the 2- and 4-monoacetates in the ratio 3 : 2, whereas with acetic anhydride–pyridine the ratio was 89 : 11, in agreement with related work which has shown that the C-4 hydroxy-group is less reactive than that at C-2. Deacetylation of the 2,4-diacetate on alkaline alumina gave the 4-acetate and the diol (123) but none of the 2-acetate. In this work the positions of the free hydroxyls in the monoesters were located by methanesulphonylation followed by S_N2 displacements to give identifiable compounds.[209]



(122)



(123)



(124)

In analogous work, partial acetylation of (124) with acetic anhydride in pyridine gave the 4- and 2-esters in the ratio 36 : 65, whereas acetyl chloride in pyridine gave a ratio 1 : 19. Again partial deacetylation of the diacetate gave only the 4-monoester.[210]

Full details have appeared on the partial acetylation of a series of nucleosides with both 2′- and 3′-hydroxy-groups free. In all cases esterification was favoured at the 2′-position; however, after equilibration the 3′-esters predominated.[211]

[206] C. Pedersen, *Acta Chem. Scand.*, 1968, **22**, 1888.
[207] N. Roy and T. E. Timell, *Carbohydrate Res.*, 1968, **7**, 82.
[208] C. P. J. Glaudemans and H. G. Fletcher jun., *Carbohydrate Res.*, 1968, **7**, 480.
[209] K. Čapek, J. Šteffkova, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 781.
[210] K. Čapek, J. Šteffkova, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 1750.
[211] G. A. R. Johnston, *Tetrahedron*, 1968, 6987.

Acetylation of β-maltose monohydrate with acetyl chloride in pyridine–toluene gave a product having one free hydroxy-group shown to be at C-3 by the characterisation of 3-*O*-methyl-D-glucitol after methylation, deacetylation, reduction, and methanolysis.[58]

The exchange occurring when penta-*O*-acetyl-α- and β-D-mannopyranose were heated in labelled acetic acid ($Me^{14}CO_2H$) has been studied. Surprisingly, it was reported that exchange was not accompanied by a change in optical rotation (indicative of anomerisation). In the case of the α-anomer, 2% of the total exchange was determined to have occurred at positions other than C-1. No exchange occurred with methyl tetra-*O*-acetyl-D-mannopyranosides.[212] In a continuation of this study the exchanges occurring when the penta-*O*-acetyl-D-galactofuranoses and -pyranoses were similarly treated were examined and discussed.[213]

Substituted Acetates and Other Nonaromatic Carboxylates

Reaction of carbohydrate hydroxy-groups with reagent (125) was reported to give chlorofluoroacetates which can be identified by characteristic ^{19}F–^{1}H n.m.r. doublets.[214] From work with nucleoside derivatives it has been shown that the methoxyacetyl and phenoxyacetyl groups are of value as protecting groups of very high alkaline lability.[215]

Solutions of glucose in aqueous formic acid were shown to give chromatographically separable products believed to be formate esters. Lactic, acetic, and chloroacetic acids did not cause esterification under the same conditions; the dangers of analysing sugars in solutions containing formic acid are apparent.[216]

(125)

(126)

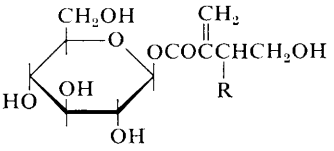
Russian workers, in particular, have shown interest in aminoacyl derivatives. Preparation of such derivatives at the 2′- and 3′- positions of nucleotides was accomplished using the imidazole derivative (126); acid hydrolysis gave α-aminopropionyl esters.[217] The acid-catalysed removal of aminoacyl residues from C-6 of 2-amino-2-deoxy-aldoses and from C-9 of the methyl ester of neuraminic acid have been studied at a variety of acid strengths.[218]

[212] J. Świderski and J. Struciński, *Roczniki Chem.*, 1968, **42**, 1051.
[213] J. Świderski and J. Struciński, *Roczniki Chem.*, 1968, **42**, 1295.
[214] L. D. Hall and L. Evelyn, *Chem. and Ind.*, 1968, 183.
[215] C. B. Reese and J. C. M. Stewart, *Tetrahedron Letters*, 1968, 4273.
[216] T. McCullough, E. DeJong, and C. Caster, *J. Chromatog.*, 1968, **37**, 545.
[217] A. A. Kraevskii, P. P. Purygin, L. Rudzite, Z. S. Belova, and B. P. Gottikh, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 378.
[218] V. A. Derevitskaya and V. M. Kalinevich, *Khim. prirod. Soedinenii*, 1968, **4**, 28.

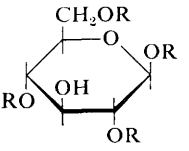
The synthesis of 6-*O*-methacroyl-D-galactose has been described *via* the 1,2:3,4-di-*O*-isopropylidene derivative, and it has been used in polymerisation studies.[219]

During investigations of the synthesis of surface-active derivatives of glucose various esterifications were examined, and it was observed, as was to be expected, that the primary hydroxyls esterified most readily, and of the secondaries, the anomeric and that at C-2 were the most reactive.[220] Similarly, selective acylation of D-fructose showed that, with low proportions of acyl chlorides (benzoyl, caproyl, lauryl, and palmityl), the 1-esters were obtained preferentially and with more reagent 1,2-diesters were produced. Other partially substituted derivatives were prepared during this work and, in some cases, were converted into acetal derivatives; the surface active properties were investigated.[221] Such properties of compounds obtained from sucrose by ester exchange from methyl palmitate were also examined,[222] and the dependence of the degree of substitution occurring during the similar transfer from methyl stearate on the reaction conditions was explored.[223] Sucrose was treated with sulphonyl chlorides and alkali-metal isocyanates to give *N*-alkylsulphonylurethanes which also had surface-active properties.[224]

New naturally occurring compounds found are three diesters of trehalose which have been isolated from *M. fortuitum*; one contained two tuberculostearic acid moieties, and the others one of this and one of either stearic acid or palmitic acid.[225] From *Tulipa gesnerania*, compounds having antibiotic activity have been isolated and found to have structures (127) and



(127) R = H
(128) R = OH



(129) R = $COCH_2CH_2NO_2$

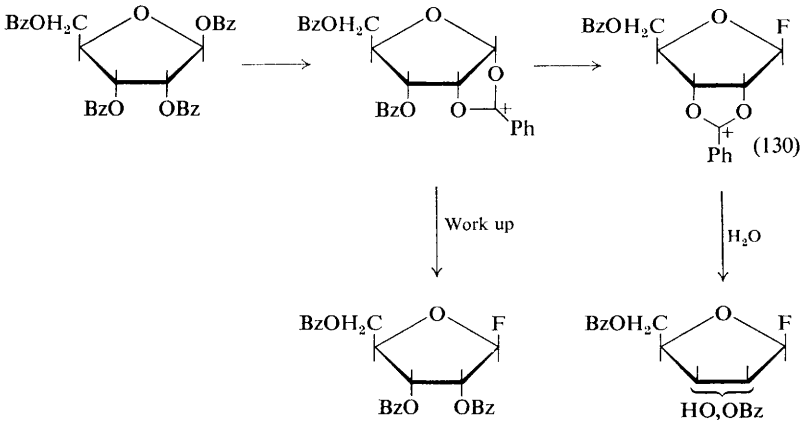
(128). Inactive isomers, shown to be the 6-substituted compounds, were also isolated.[226] Hiptagin, isolated from the root-bark of *Hiptage madablota* was shown to be the 1,2,4,6-tetra-*O*-(3-nitropropionyl)-β-D-glucopyranose (129), and thus to be identical with 'endecaphyllin X'. On

[219] W. A. P. Black, J. A. Colquhoun, and E. T. Dewar, *Makromol Chem.*, 1968, **117**, 210.
[220] E. Reinefeld and H. F. Korn, *Stärke*, 1968, **20**, 181.
[221] E. Reinefeld and S. K. Laudianos, *Zucker*, 1968, **21**, 236.
[222] M. Bares and J. Zajic, *Sb. Vys. Sk. Chem.-Technol. Praze, Potraviny*, 1967, **15**, 89.
[223] M. Ranny, J. Haumer, and J. Novak, *Tenside*, 1968, **5**, 40.
[224] W. Gerhardt, *Tenside*, 1968, **5**, 10.
[225] E. Vilkas, A. Adam, and M. Senn, *Chem. Phys. Lipids*, 1968, **2**, 11.
[226] R. Tschesche, F.-J. Kämmerer, and G. Wulff, *Tetrahedron Letters*, 1968, 701.

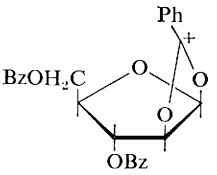
methylation it afforded the same product as that prepared by esterification of 3-*O*-methyl-D-glucose.[227]

Benzoates

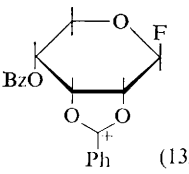
Studies on the reaction of carbohydrate esters in hydrogen fluoride have been extended in an examination of ribo- and arabino-furanosyl derivatives. Ribofuranosyl compounds reacted as illustrated in Scheme 10. Esterified D-arabinofuranose derivatives initially gave ion (131) which slowly reacted to give (130). Anomeric configurations were assigned by considering $J_{1,2}$ values, and glycosyl fluorides were detected by observing 58 Hz geminal fluorine–proton coupling. Further, n.m.r. spectroscopy was used to follow the reaction in detail and to detect both neutral and charged species.[228] Similarly, the ribopyranosyl benzoates gave 1,2-benzoxonium ions, and the arabinopyranose analogues rearranged to give the ion (132) which then ring-contracted to give a furanosyl product which was ultimately also obtained from tetra-*O*-benzoyl-D-ribopyranose.[206]



Scheme 10



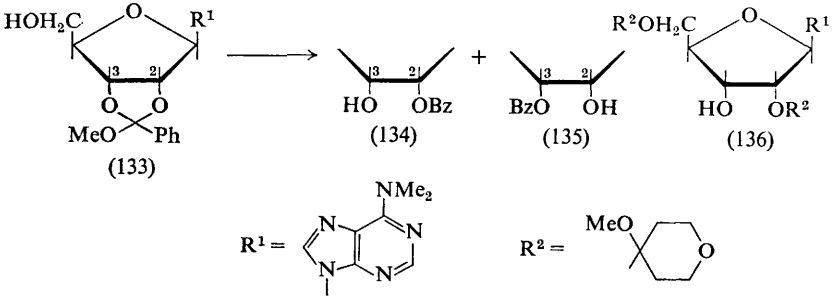
(131)



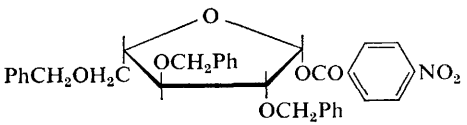
(132)

[227] R. A. Finnegan and R. A. Stephani, *J. Pharm. Sci.*, 1968, **57**, 353.
[228] N. Gregersen and C. Pedersen, *Acta Chem. Scand.*, 1968, **22**, 1307.

Treatment of 2′,3′-*O*-methoxybenzylidene-N^6-dimethyladenosine (133) with 80% acetic acid gave (134) and (135) in the ratio 1 : 10; after equilibrium the ratio was 3·3 : 1. The partially blocked nucleoside (136) was obtained [229] from (135) by acetalation, followed by deacylation.



Several substituted benzoyl esters of methyl glucopyranosides have been described,[116] and attention has been drawn to the possible reduction of *p*-nitrobenzoate esters to *p*-nitroso-analogues during attempted removal of benzyl ethers by hydrogenolysis. Reduction of (137) gave the fully substituted nitroso-derivative.[155]



(137)

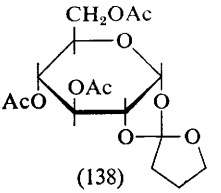
Partial acid hydrolysis of 1,2,3,5-tetra-*O*-benzoyl-4,6-*O*-ethylidene-D-glucitol gave the 1,2,4,6-tetra-ester, formed by ester migration.[230]

Carboxylic Orthoesters

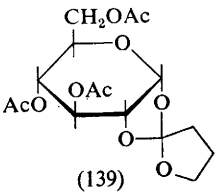
Reference is made on p. 10 to the important application of glycosyl 1,2-orthoesters in glycoside synthesis, and it has now been shown that they also provide suitable precursors for 1,2-alkylidene-aldoses.[186] With butyrolactone the orthoesters (138) and (139) were obtained in almost equal proportions.[186]

Diastereoisomeric nitrogen-containing orthoester analogues (140) were obtained on treatment of 1,2-*O*-isopropylidene-α-D-xylofuranose with reagent (125);[214] this represents a divergence from the usual mode of

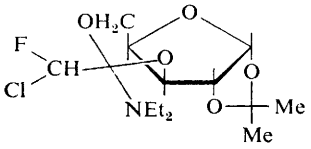
[229] D. P. L. Green and C. B. Reese, *Chem. Comm.*, 1968, 729.
[230] M. Matsui, M. Okada, and M. Ishidate, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1288.



(138)



(139)

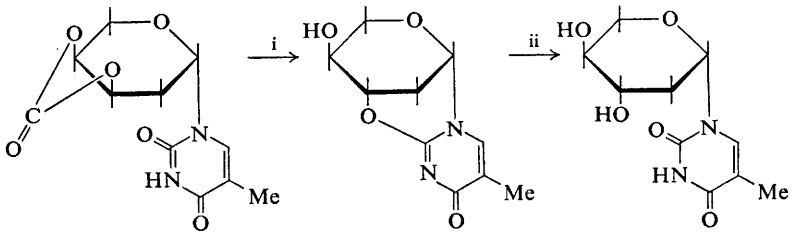


(140)

action of this reagent (see p. 48). The use of orthoesters in the synthesis of partially substituted nucleosides is referred to on p. 51.

Carbonates

A carbonate ester has been used as shown in Scheme 11 in an interesting configuration inversion reaction.[231]



Reagents: i, heat, py; ii, OH^-

Scheme 11

The use of the β,β,β-tribromoethoxycarbonyl group [which with alcohols gives tribromoethyl carbonates ($Br_3CCH_2OCO \cdot OR$)] for protective purposes in nucleoside reactions has been assessed, and alternative groups used for this purpose have been briefly reviewed. Of particular interest was the observation that the new derivatives were reconverted to the parent alcohols by use of a zinc–copper couple, and at a rate in excess of that of the corresponding trichloro-analogues. The esters were prepared readily by use of β,β,β-tribromoethyl chloroformate, and primary hydroxy-groups showed marked selectivity; secondary groups will, however, esterify. With ribonucleosides complications are introduced by the formation

[231] G. Etzold, R. Hintsche, and P. Langen, *Z. Chem.*, 1968, **8**, 61.

of 2′,3′-cyclic carbonates, and it was also observed that 2′,3′-*O*-isopropylideneadenosine underwent substitution mainly at the amino-group although some 5′-ester was obtained.[232]

The esters, believed to have structure (141), formed on introduction of carbon dioxide into alkaline solutions of carbohydrates, have been

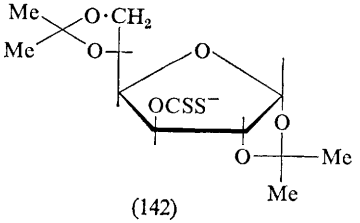
$$RO \cdot CO_2^- M^+$$

(141)

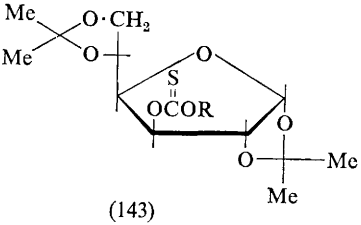
examined by electrophoretic methods; such esters were not formed from polyhydroxy-compounds in solutions containing inorganic carbonates. It was suggested that reaction occurs, where possible, at primary sites, and that only monosubstituted products were obtained. Polyols apparently formed more stable esters than did free sugars.[233] Carbonates are also referred to in the following section.

Thiocarbonates

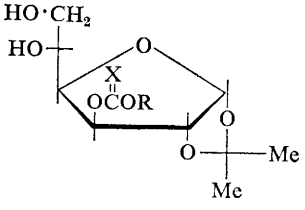
A new rapid method has been described for the preparation of bis-(1,2:5,6-di-*O*-isopropylidene-3-*O*-thiocarbonyl-α-D-glucofuranose)disulphide (142). Di-isopropylidene-D-glucose was treated with aqueous sodium hydroxide and carbon disulphide in DMSO and after acidification the xanthate



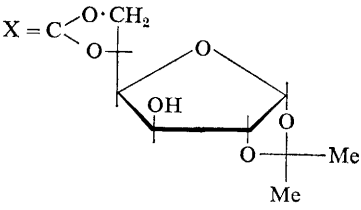
(142)



(143)
R = Me, Et, Pr, Bu or Ph



(144) X = S
(145) X = O
(146) X = H_2



(147) X = S
(148) X = O

[232] A. F. Cook, *J. Org. Chem.*, 1968, **33**, 3589.
[233] J. L. Frahn, *Austral. J. Chem.*, 1968, **21**, 811.

formed was oxidised with iodine.[234] The resulting compound (142) underwent solvolysis with alcohols and phenols in pyridine to give esters of the type (143), from which, by partial hydrolysis, esters (144) were obtained. These, in the presence of triethylamine, rearranged to (147). Ester (144) with lead tetra-acetate or silver nitrate gave oxygen analogues (145), and with hydrogen over a nickel catalyst were converted to the acetal (146). The carbonate (145) also rearranged in basic conditions to give (147).[235]

Phosphates and Phosphites

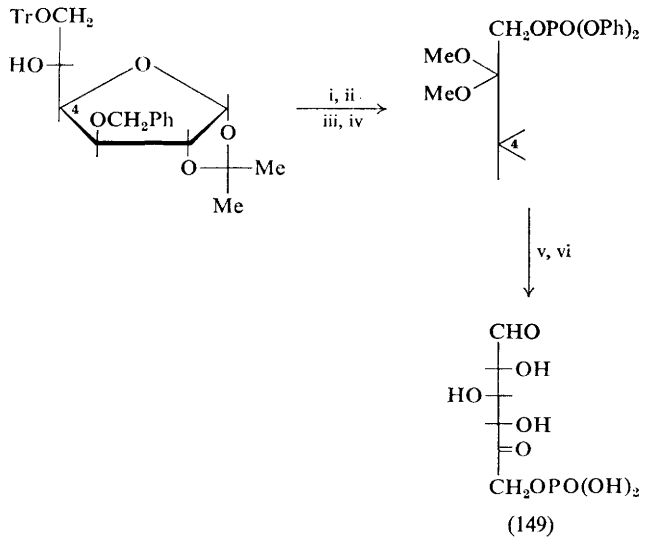
Phosphate esters groups have been introduced, for the first time, on to primary hydroxy-groups by treatment of toluene-*p*-sulphonate esters with lithium diphenyl phosphate in refluxing DMF; the method should have general applicability. By the use of otherwise acetylated derivatives D-glucose 6-phosphate was obtained in low yield, and the corresponding methyl α- and β-glycosides in *ca.* 35% yield after standard reactions to remove the protecting groups.[236]

Sugar Phosphates and Phosphites.—The synthesis and reactions of phosphorylated deoxy-sugars have been reviewed.[237] Amongst specific esters, α-L-rhamnopyranosyl phosphate was prepared by fusion of the tetra-acetate with anhydrous phosphoric acid.[238] A convenient synthesis of D-glucopyranose 1,6-diphosphate has been developed by converting the 6-phosphate disodium salt into the β-tetra-acetyl dihydrogen phosphate, which on treatment with anhydrous phosphoric acid, followed by deacetylation gave the required diester in *ca.* 18% overall yield.[239] *o*-Nitrophenyl β-D-galactopyranoside 6-phosphate, a substrate for staphylococcal β-D-galactosidase, was prepared by way of the 2,3,4-tri-*O*-trimethylsilyl ether (obtained by specific partial hydrolysis of the tetra-ether).[240]

The synthesis of D-*xylo*-hexos-5-ulose 6-phosphate (149), considered to be an intermediate in the biochemical conversion of D-glucose into *myo*-inositol, has been accomplished as shown in Scheme 12.[241]

D-[1-^{14}C]Mannitol 1-phosphate and D-mannitol 1-[^{32}P]phosphate were prepared by treatment of mannitol with polyphosphoric acid containing appropriate labels.[242] Hydrolysis of the glycolipid of the shellfish *Corbicula sandai* gave D-mannose 6-phosphate as the phosphate component. This is

[234] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1968, **7**, 99.
[235] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1968, **6**, 34.
[236] A. K. Chatterjee and D. L. MacDonald, *J. Org. Chem.*, 1968, **33**, 1584.
[237] L. Szabo, *Adv. Chem. Ser.*, *No.* 74, 1968, 70.
[238] A. K. Chatterjee and D. L. MacDonald, *Carbohydrate Res.*, 1968, **6**, 253.
[239] K. W. Buck, *Carbohydrate Res.*, 1968, **6**, 247.
[240] W. Hengstenberg and M. L. Morse, *Carbohydrate Res.*, 1968, **7**, 180.
[241] D. E. Kiely and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 3723.
[242] E. T. McGuinness and J. L. Beebe, *J. Labelled Compounds*, 1968, **3**, 419.



(149)

Reagents: i, DMSO, Ac_2O; ii, aq. AcOH; iii, $HC(OMe)_3$; iv, $(PhO)_2POCl$; v, H_2–Pt; vi, H^+

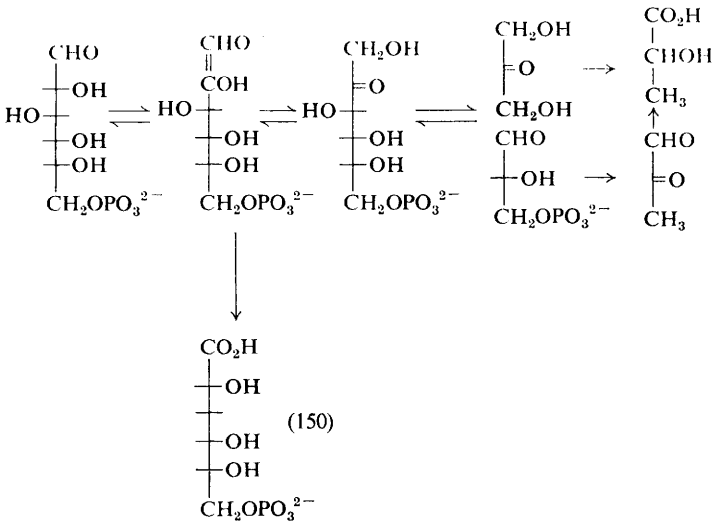
Scheme 12

believed to be the first time that this ester has been found as a component of a glycolipid.[243]

The hydrolyses of D-fructose-1-phosphate and -1,6-diphosphate have been examined at different pH values, and it was observed that fragmentation of the carbon chain occurred concurrently with cleavage of the ester bonds.[244] In related work the alkaline reaction of D-glucose 6-phosphate (*cf.* Vol. 1, p. 65) has been investigated in detail. Initially the dianion equilibrates with the enol conjugate base which rearranges partially and irreversibly to D-glucometasaccharinic acid 6-phosphate and gives, in part, D-fructose 6-phosphate which undergoes reverse aldol reaction to D-glyceraldehyde 3-phosphate and dihydroxyacetone. These finally afford lactic acid and orthophosphate (Scheme 13). No glucose was produced in the reaction so no direct ester hydrolysis was deemed to have occurred. The similarity between these degradations and the anaerobic metabolism of carbohydrates was noted.[245]

The product from the previously described Ruff degradation of compound (150) has been shown to be a 4 : 1 mixture of the 4- and 5-phosphates of 2-deoxy-D-*erythro*-pentose; *i.e.* extensive migration of the phosphate (surprisingly away from the primary alcoholic group) had occurred.[246]

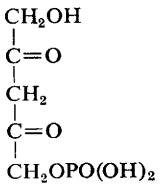
[243] O. Itasaka, *J. Biochem.* (*Japan*), 1968, **63**, 347.
[244] N. V. Volkova, I. I. Semenyuk, and A. A. Yasnikov, *Ukrain. khim. Zhur.*, 1967, **33**, 712.
[245] C. Degani and M. Halmann, *J. Amer. Chem. Soc.*, 1968, **90**, 1313.
[246] F. Trigalo, P. Szabó, and L. Szabó, *J. Chem. Soc.* (*C*), 1968, 901.



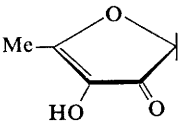
(150)

Scheme 13

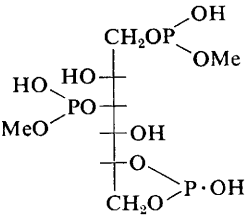
The influences of spinach leaf chloroplast isomerase on D-ribose 5-phosphate were investigated by various physical means; it was concluded that the product was (151) rather than D-*erythro*-pentulose 5-phosphate,[247] and from the same ribose ester, compound (152) was obtained in 45% yield after heating with sodium acetate in aqueous acetic acid.[248] Hexose



(151)



(152)



(153)

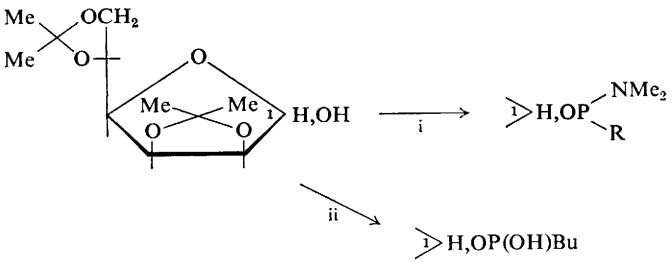
phosphates have been exposed to neutron irradiation and the resulting ^{32}P recoil products were shown to be phosphorous and phosphoric acids which were independent of the chemical form of the target.[249]

A reference to the examination of $^{31}P,^{1}H$ coupling constants of some sugar phosphate derivatives is given on p. 197.

D-Mannitol with triethyl phosphite gave a fully substituted tricyclic ester which, from i.r. evidence, had the 1,2:3,4:5,6 structure. In the

[247] F. C. Knowles and N. G. Pon, *J. Amer. Chem. Soc.*, 1968, **90**, 6536.
[248] H. G. Peer and G. A. M. van den Ouweland, *Rec. Trav. chim.*, 1968, **87**, 1017.
[249] Y. Kiso, M. Kobayashi, Y. Kitaoka, K. Kawamoto, and J. Takada, *Bull. Chem. Soc. Japan*, 1968, **41**, 1642.

course of the work, 1,2:3,4:5,6-tri-*O*-isopropylidenemannitol, 1,2:5,6-di-*O*-isopropylidenemannitol 3,4-ethyl phosphite, and 3,4-*O*-isopropylidenemannitol 1,2:5,6-bis(ethyl phosphite) were also examined.[250] *trans*-Esterification of dimethyl phosphite to D-mannitol in the presence of catalytic amounts of sodium gave an oily product soluble in water and in organic solvents which was believed to be a polymer of (153).[251] Other Russian work has led to the production of various glycosyl phosphites as shown in Scheme 14.[251a]



Reagents: i, $(Me_2N)_2PR$ ($R = Me_2N$ or Me); ii, $(BuPO)_2$

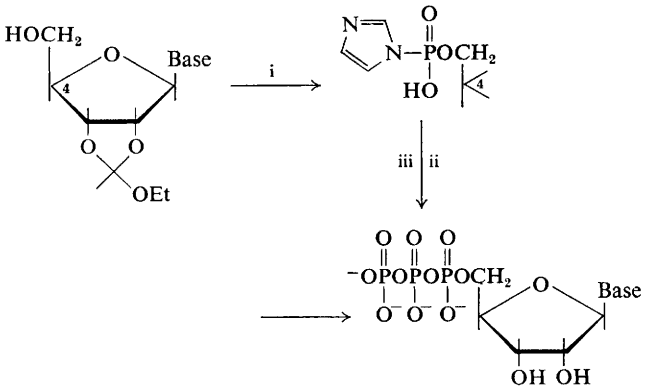
Scheme 14

Nucleoside Phosphates.—The syntheses of compounds of this series illustrate the application of a variety of phosphorylating reagents. Treatment of uridyl and thyminyl ribofuranosides in pyridine solution with bis-(β,β,β-trichloroethyl)phosphorochloridate caused selective esterification at the 5′-position, and the 5′-nucleotide derivatives were obtained in 40–70% yield. Removal of the blocking groups was readily achieved using zinc dust.[252] The 5′-phosphate of the nucleoside analogue 1-β-D-ribofuranosylindole has been prepared with the aid of diphenylphosphoryl chloride,[253] and the β-cyanoethyl phosphate method was applied to 1-β-D-arabinofuranosyl pyrimidines.[254] Phosphoryl chloride has been used without organic solvents in the 5′-phosphorylation of 2′,3′-*O*-isopropylidene nucleosides,[255] and it has been shown that esterification of such compounds with the same reagent was accompanied by side reactions including the formation of cyclonucleosides, 5′-chloro-5′-deoxy compounds and the degradation of purine nuclei.[256] Phosphorylation can also be

[250] O. V. Voskresenskaya, N. A. Makarova, P. A. Kirpichnikov, and E. T. Mukmenev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 1393.
[251] E. A. Kirichenko, T. G. Shestakova, K. I. Shchekina, and E. E. Nifant'ev, *Trans. Mosk. Khim.-Tekhnol. Inst.*, 1967, 112.
[251a] I. P. Gudkova, I. K. Golovnikova, and E. E. Nifant'ev, *Zhur. obshchei Khim.*, 1968, **38**, 1340.
[252] A. Franke, K.-H. Scheit, and F. Eckstein, *Chem. Ber.*, 1968, **101**, 2998.
[253] M. N. Vigdorchik, M. N. Preobrazhenskaya, and N. N. Suvorov, *Tetrahedron Letters*, 1968, 4645.
[254] M. Privat de Garilhe, *Bull. Soc. chim. France*, 1968, 1495.
[255] M. Yoshikawa and T. Kato, *Bull. Chem. Soc. Japan*, 1967, **40**, 2849.
[256] K. Kusashio and M. Yoshikawa, *Bull. Chem. Soc. Japan*, 1968, **41**, 143.

accomplished enzymically. For example, 1-β-D-arabinofuranosyl-5-fluorouracil and -5-fluorocytosine were esterified by enzyme-catalysed transfer from *p*-nitrophenyl phosphate with *ca.* 30% efficiency. A chemical method was applied with less efficiency (10% overall) but was found to be more suitable for large-scale work. The nucleotides retained the anti-tumour activity of the original nucleosides, but the phosphorylation reduced this activity slightly rather than enhancing it.[257]

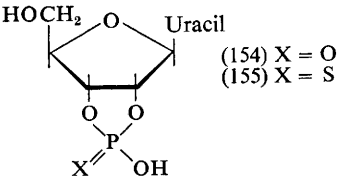
Triphosphates of nucleosides were prepared as illustrated in Scheme 15. Diphosphates were also produced, and the reaction was also applied to a 2-deoxynucleoside with the 3′-hydroxy-group protected by acetylation.[258]



Reagents: i, $\left(\text{N}\!=\!\text{C}\!-\!\text{N}\right)_3$—PO; ii, PO_4^{3-}; iii, H^+

Scheme 15

2′,3′-Cyclic phosphates of nucleosides have been synthesised by treatment of free nucleosides with triethyl phosphite and an acid catalyst, followed by oxidative cyclisation;[259] similarly a cyclic derivative (154) was obtained by treating 5′-*O*-acetyluridine with tri-imidazolylphosphine



(154) X = O
(155) X = S

[257] W. Strider, C. Harvey, and A. L. Nussbaum, *J. Medicin. Chem.*, 1968, **11**, 524.
[258] K.-H. Scheit, *Chem. Ber.*, 1968, **101**, 1141.
[259] A. Holý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 2245.

sulphide in pyridine followed by deacetylation. In addition, the sulphur-containing analogue (155) was obtained. A mechanism for the reaction was proposed and compound (155) was briefly studied.[260]

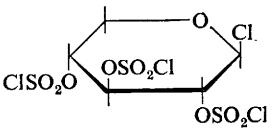
Several papers have appeared on the phosphorylation of nucleosides under possible primitive earth conditions. Uridine was found to be phosphorylated with inorganic phosphates in aqueous solution in the presence of cyanogen, cyanoformamide, cyanate, cyanamide, thioformate, ethyl isocyanide, and a water-soluble carbodi-imide.[261] Orthophosphates themselves are not known to be effective phosphorylating agents but on heating they give condensed phosphates which are effective [262] and esterify adenosine in aqueous solution over a wide pH range.[263] A further reagent, cyanovinyl phosphate prepared by the reaction of phosphate with cyano-acetylene, has been shown to phosphorylate uridine at the 5′-position in aqueous solution.[264]

The use of the phenylboronate blocking group in 5′-phosphorylations is mentioned on p. 74.

Sulphates

D-Glucose 2-sulphate, D-galactose 2- and 3-sulphate, and D-galactose 2,3-disulphate have been synthesised by well-defined routes. Periodate oxidation studies showed that in contrast to other sulphates which are oxidised in the pyranose ring form, 2-sulphates react in the open-chain modification. Comments were made on the utility of i.r. spectroscopy for the assignment of the positions of sulphate groups. 2-Esters were found to be particularly anomalous.[265]

Treatment of D-xylose with sulphuryl chloride and pyridine in chloroform solution gave the esterified glycosyl chloride (156) in good yield, and



(156)

from this the crystalline α-isomer was obtained after anomerisation. Both anomers on methanolysis gave largely the glycosides formed by inversion of configuration indicating that the chlorosulphate groups did not participate in the displacements.[87]

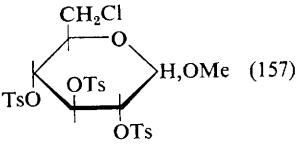
[260] F. Eckstein and H. Gindl, *Chem. Ber.*, 1968, **101**, 1670.
[261] R. Lohrmann and L. E. Orgel, *Science*, 1968, **161**, 64.
[262] J. Rabinowitz, S. Chang, and C. Ponnamperuma, *Nature*, 1968, **218**, 442.
[263] A. Schwartz and C. Ponnamperuma, *Nature*, 1968, **218**, 443.
[264] J. P. Ferris, *Science*, 1968, **161**, 53.
[265] S. Peat, D. M. Bowker, and J. R. Turvey, *Carbohydrate Res.*, 1968, **7**, 225.

Sulphonates

The first part of a two-part review on sulphonates has been published, covering synthesis, relative reactivity of hydroxy-groups, physical properties, chemical stability, reaction with LAH, and with some alkaline reagents.[266]

Synthesis.—Monotoluene-*p*-sulphonylation of methyl 6-*O*-trityl-α-D-mannofuranoside gave the 2-toluene-*p*-sulphonate in 84% yield, which by standard sequences was converted into 3,5-di-*O*-methyl-D-mannose;[150] this was characterised chemically and shown to be different from a compound previously claimed to have this structure. Partial methanesulphonylation of methyl 3-acetamido-3,6-dideoxy-α-D-mannopyranoside (124) gave some disulphonyl derivative in addition to the 2-ester.[210] Partial toluene-*p*-sulphonylation of 5′-*O*-acetyl-6-azauridine gave the 3′-ester.[267]

The sulphonylation of methyl α- and β-D-glucopyranosides has been studied at various temperatures, and the products characterised by n.m.r. and mass spectrometry. It was shown that the compounds described (K. Hess and H. Stenzel, *Ber.*, 1935, **68**, 981) as methyl 4-chloro-4-deoxy-2,3,6-tri-*O*-toluene-*p*-sulphonyl-α- and -β-D-glucopyranosides were in fact the 6-chloro-6-deoxy-2,3,4-triesters (157); these compounds were obtained using



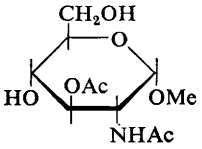
(157)

toluene-*p*-sulphonyl chloride and pyridine at 75°. A second compound obtained from methyl α-D-glucopyranoside, or from (157, α-anomer), described previously (Hess and Stenzler, *ibid.*) as methyl 4,6-dichloro-4,6-deoxy-2,3-di-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside was shown to be the corresponding *galacto*-isomer, *i.e.* inversion had occurred at C-4. Toluene-*p*-sulphonylation of either methyl α- or β-D-glucopyranoside with the above reagents at 27° for 16 days gave, in the latter case, predominantly the tetra-ester, whereas the former gave a mixture of the tetra-ester and (157, α-anomer). Methanesulphonylation at 75° of methyl α-D-glucopyranoside gave similar products but the β-anomer gave the 6-chloro-6-deoxy-2,3,4-tri-*O*-methanesulphonyl derivative together with the 4,6-dichloro-4,6-dideoxy-2,3-di-*O*-methanesulphonyl-β-D-glucopyranoside, but no *galacto*-isomer.[268] In a further paper, conditions have been

[266] D. H. Ball and F. W. Parrish, *Adv. Carbohydrate Chem.*, 1968, **23**, 233.
[267] J. Beránek and F. Šorm, *Coll. Czech. Chem. Comm.*, 1968, **33**, 901.
[268] F. W. Parrish, F. H. Bissett, M. E. Evans, M. L. Bazinet, W. Yeomans, and L. Long jun., *Carbohydrate Res.*, 1968, **6**, 503.

described for the efficient substitution of the primary hydroxy-group of hexopyranosides by chlorine using methanesulphonyl chloride in DMF; the acid fluoride did not give fluoro-derivatives.[269]

It is of further interest that in the methanesulphonylation of methyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside (158) at room temperature, the 4,6-di-ester was accompanied by the 6-chloro-6-deoxy compound, showing that in this case the 6-methanesulphonyl group was displaced by pyridinium hydrochloride even in the cold.[270]



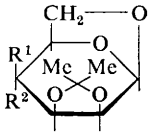
(158)

Displacement Reactions without Participation.—Direct displacements using hydrazine or azide ion have been used in several syntheses of amino-sugars (see Section 8, p. 80), and they will not be discussed here. Factors affecting the displacement of sulphonyloxy-groups have been discussed, attention being drawn to the hindrance of a *β-trans*-axial substituent on an aldopyranose ring to the rearward approach of a nucleophile. The difficulty in displacing sulphonyloxy-groups on C-2 was also discussed in terms of the dipolar interactions present in the transition state.[271] (For a reported direct displacement of a C-2 group see p. 175.)

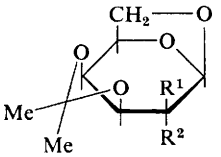
In a most interesting paper, Horton and his colleagues[272] have shown that the sulphonyloxy-groups cannot be displaced from any of the compounds (159)–(162) (two epimeric pairs), using sodium or lithium azide in DMF or DMSO for 2–7 days; starting material was recovered in high yield. This non-reactivity was attributed to the extreme steric hindrance to the formation of the transition states (159a)–(162a). Reaction of (160) with anhydrous hydrazine, which will often displace sulphonyl esters not reactive towards azide ion, also gave only a high recovery of starting material.

Treatment of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-gulofuranose (163) with tetra-n-butylammonium fluoride in acetonitrile at 60° gave a mixture of the 3-deoxy-3-fluoro-derivative (164) and the unsaturated sugar (165). Compound (164) was separated from the mixture after the hydrogenation of the double bond in (165), and the acetal groups were removed to give 3-deoxy-3-fluoro-D-galactose (166), characterised as its tetra-acetate.[273] This reaction contrasts with that of the *allo*-isomer of

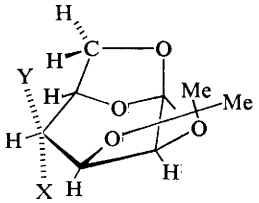
[269] M. E. Evans, L. Long jun., and F. W. Parrish, *J. Org. Chem.*, 1968, **33**, 1074.
[270] J. Hill and L. Hough, *Carbohydrate Res.*, 1968, **8**, 398.
[271] Y. Ali and A. C. Richardson, *J. Chem. Soc.* (*C*), 1968, 1764.
[272] A. K. Chatterjee, D. Horton, and J. S. Jewell, *Carbohydrate Res.*, 1968, **7**, 212.
[273] J. S. Brimacombe, A. B. Foster, R. Hems, and L. D. Hall, *Carbohydrate Res.*, 1968, **8**, 249.



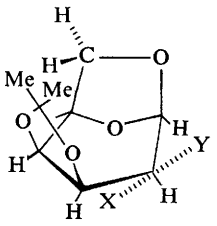
(159) R^1 = Ts, R^2 = H
(160) R^1 = H, R^2 = Ts



(161) R^1 = Ts, R^2 = H
(162) R^1 = H, R^2 = Ts

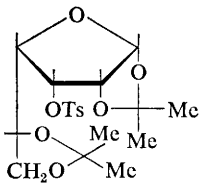


(159a) X = N_3^-, Y = Ts
(160a) X = Ts, Y = N_3^-

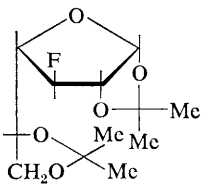


(161a) X = N_3^-, Y = Ts
(162a) X = Ts, Y = N_3^-

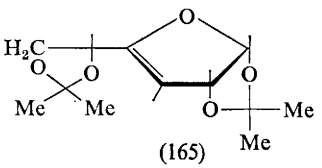
(163) (Vol. 1, p. 85), which gave displacement with no reported elimination. The same group have also treated (163) with azide and with benzoate ions (with no reported accompanying elimination).[274] The former reaction provides a new route to 3-acetamido-3-deoxy-D-galactose derivatives. The above displacement on (163) re-emphasises the ready displacement of *endo*-sulphonyloxy-groups.



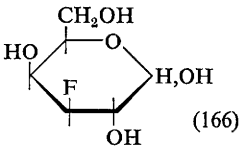
(163)



(164)



(165)



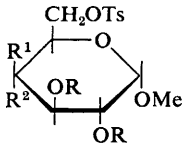
(166)

These results are in marked contrast with the unreactivity of the 3-*exo*-sulphonyloxy-group in the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose derivative. This unreactivity has been further emphasised by the observation that on heating the 3-*O*-bromobenzenesulphonate with dimethylamine, the

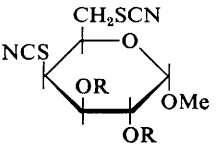
[274] J. S. Brimacombe, P. A. Gent, and M. Stacey, *J. Chem. Soc.* (*C*), 1968, 567.

main reaction was attack on the aromatic ring and displacement of the bromo-group by dimethylamine.[275]

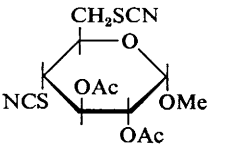
Displacement reactions on methyl 2,3-di-*O*-acetyl- and 2,3-di-*O*-benzoyl-4,6-di-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside (167) and -galactopyranoside (168) have been described. Displacement with azide ion gave the 4,6-diazido-4,6-dideoxy-derivatives with *galacto*- and *gluco*-configurations respectively; reduction gave the corresponding diamino-dideoxy derivatives.[276] Reaction of (167) with potassium thiocyanate in DMF gave the 4,6-dideoxy-4,6-dithiocyano-galactoside (169). In the case of the di-*O*-acetyl compound (167) the 4,6-dideoxy-4,6-dithiocyano compound with the *gluco*-configuration (170) was formed as a by-product.[277] This was a significant observation as it showed that participation of the



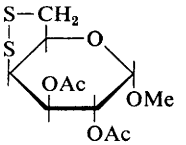
(167) R^1 = H, R^2 = Ts
(168) R^1 = Ts, R^2 = H
R = Ac or Bz



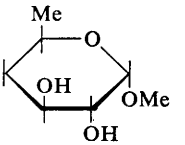
(169) R = Ac or Bz



(170)



(171)

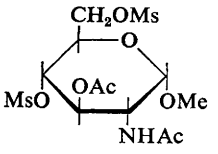


(172)

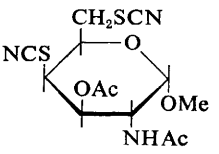
C-3-acetoxy-group was occurring in the displacement of the C-4-toluene-*p*-sulphonyloxy function. The *gluco*-derivative (170) was also prepared by displacement on the di-*O*-acetyl *galacto* compound (168). Base-catalysed hydrolysis of (169) gave the 4,6-disulphide (171) which arose *via* the 4,6-dithio-compound. Desulphurisation of (169), (170), or (171) gave 4,6-dideoxy-D-*xylo*-hexopyranose (172) after de-esterification and acid hydrolysis.

Hough's group have extended the above study to the analogous dimethanesulphonate (173), which with potassium thiocyanate in DMF gave the 4,6-dithiocyano-*galacto*-derivative (174) together with the disulphide (175); desulphurisation of either gave the 2-acetamido-2,4,6-trideoxy-derivative (176).[270] Treatment of (173) with sodium benzoate in DMF gave (177), which after de-esterification gave methyl 2-acetamido-2-deoxy-α-D-galactopyranoside, a convenient route to this substance.

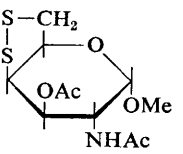
[275] D. Horton, J. S. Jewell, and H. S. Prihar, *Canad. J. Chem.*, 1968, **46**, 1580.
[276] J. Hill, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1968, **8**, 7.
[277] J. Hill, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1968, **8**, 19.



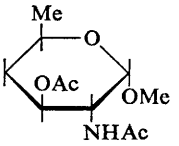
(173)



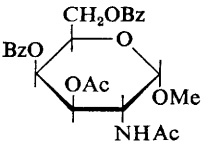
(174)



(175)



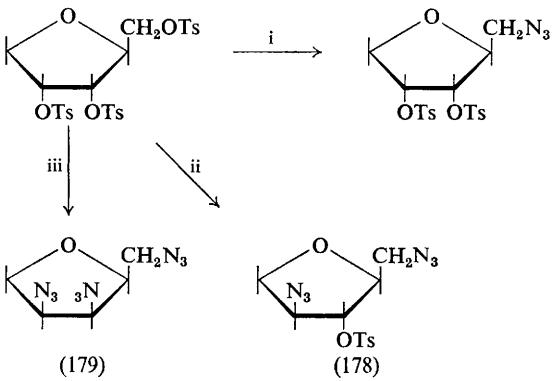
(176)



(177)

It has been shown, not unexpectedly perhaps, that the primary sulphonyloxy-group in methyl 2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside was readily displaced by azide ion, whereas the secondary one was not.[278] Displacement of the sulphonyloxy-groups in 6,6′-di-*O*-toluene-*p*-sulphonyl-α,α-trehalose with sodium azide in HMPT gave the 6,6′diazido-6,6′-dideoxy-derivative.[279]

The successive introduction of azido-groups into the 2,5-anhydropentitol skeleton has been studied as shown in Scheme 16. The structures, characterised by n.m.r., showed that in the transformation (178) to (179) an azidonium ion was not involved.[280]



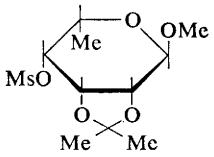
(179) (178)

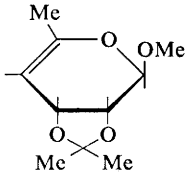
Reagents: i, NaN_3–$MeO(CH_2)_2OH$, 100°, 2 hr.; ii, NaN_3–$MeO(CH_2)_2OH$, 130°, 4 hr.; iii, NaN_3–DMF, 125°, 1·5 hr.

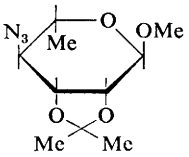
Scheme 16

[278] S. P. Dutta and A. K. Mitra, *Current Sci.*, 1968, **37**, 345.
[279] G. Birch and A. C. Richardson, *Carbohydrate Res.*, 1968, **8**, 411.
[280] J. Cleophax, J. Hildesheim, R. E. Williams, and S. D. Gero, *Bull. Soc. chim. France*, 1968, 1415.

A synthesis of 4-amino-4,6-dideoxy-L-mannose (L-perosamine) derivatives has been made in an effort to identify the natural product (which belongs to the D-series).[281] Treatment of methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranoside (180) with sodium azide in DMF gave two products in the ratio 1 : 3. The major product (181)

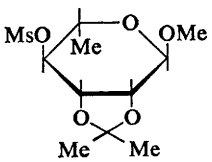

(180)

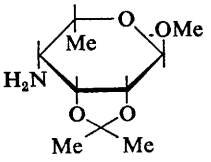

(181)


(182)

was identified by comparison with its known enantiomer; the minor product was the required 4-azido-derivative (182).[281] This was converted by standard methods into methyl 4-amino-4,6-dideoxy-L-mannopyranoside and its *N*-acetyl derivative, which were enantiomeric with methyl perosaminide and its derivative.

Full details of the reaction of methyl 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-rhamnopyranoside (183) with anhydrous hydrazine and then reduction to give the 4-amino-4-deoxy-talopyranoside derivative (184) have been given. This reaction contrasted with that of (183) with lithium azide in DMF when ring-contraction occurred (*cf.* Vol. 1, p. 79). Other products were also formed in the hydrazine reaction. The structure of (184) was established by n.m.r. and by degrading it to D-threonine.[282] The authors explained the different course of the reaction of (183) with


(183)


(184)

azide ion and with hydrazine by suggesting that because the methanesulphonyloxy-group is equatorial, the attacking charged nucleophile will interact strongly with the ring oxygen and so normal S_N2 displacement does not occur. However, the ring oxygen is situated antiparallel to the leaving group and so ring contraction occurs; for hydrazine, a neutral nucleophile, there is no hindrance to approach and so normal displacement takes place.[282] A similar explanation has been proposed by Horton,[272]

[281] J. S. Brimacombe, O. A. Ching, and M. Stacey, *Carbohydrate Res.*, 1968, **8**, 498.
[282] J. Jarý and P. Novák, *Coll. Czech. Chem. Comm.*, 1968, **33**, 1744.

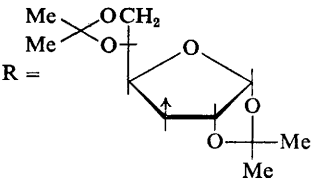
who noted that hydrazine could hydrogen-bond to (183) in a favourable position for attack. These results should be contrasted with the reaction of sodium azide in DMF on the 4-deoxy-4-iodo-analogue of (183) (see p. 83).

A method for the introduction of phosphate groups on to primary positions has been developed using the reaction of toluene-*p*-sulphonates with lithium diphenyl phosphate in DMF.[236] Treatment of 5′-*O*-acetyl-3′-*O*-toluene-*p*-sulphonyl-6-azauridine with sodium iodide in acetylacetone gave a 3′-deoxy-3′-iodo-product, which was believed to have the *xylo*-configuration and thought to have been produced by direct displacement rather than *via* cyclo-nucleoside formation.[267]

When sulphonyl derivatives were treated with basic or neutral alumina at 50°, selective hydrolysis of primary groups occurred. For example, methyl 3,4-di-*O*-methyl-2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside, methyl 4-*O*-methyl-2,3,6-tri-*O*-methanesulphonyl-α-D-mannopyranoside and methyl 2,3-di-*O*-methyl-4,6-di-*O*-methanesulphonyl-β-D-glucopyranoside all suffered loss of the 6-*O*-sulphonyl group. Compounds with only secondary sulphonyl groups were stable under the conditions.[192] When the solvents used for the hydrolysis contained alcohols, alkylation occurred at the primary site. For example, methyl 3,4-di-*O*-methyl-2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside with basic alumina in benzene or chloroform containing up to 3% of methanol gave methyl 3,4-di-*O*-methyl-2-*O*-methanesulphonyl-α-D-glucopyranoside (60%) together with its 6-*O*-methyl ether (40%). Ethyl and benzyl groups were introduced in a similar manner.[192]

Reaction of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose with tetramethylammonium hydroxide in DMSO caused smooth de-esterification, but the *p*-nitrobenzenesulphonyl analogue gave compounds (185) and (186) (54 and 10%, respectively), together with small amounts of the 3-hydroxy-derivative.[283]

(185) (186)

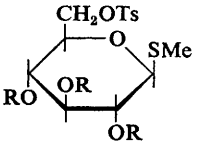


Papers describing the reactions of sulphonyl derivatives leading to unsaturated sugars are described on p. 132.

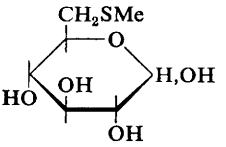
Displacements with Participation.—(a) *Sulphur functions.* Treatment of methyl 1-thio-6-*O*-toluene-*p*-sulphonyl-β-D-glucopyranoside (187) with

[283] A. Rosenthal and L. Nguyen, *Canad. J. Chem.*, 1968, **46**, 3751.

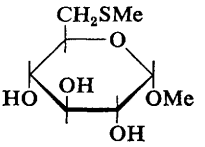
water or methanol gave 6-*S*-methyl-6-thio-D-glucopyranose (189) or its methyl α-D-glucoside (190), respectively, which meant that sulphur participation must have occurred, through the ion (191) as an intermediate.



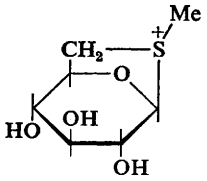
(187) R = H
(188) R = Ac



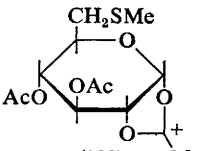
(189)



(190)



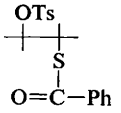
(191)



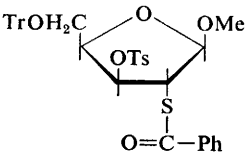
(192)

Acetolysis of the triacetate (188) gave 6-*S*-methyl-6-thio-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, formation of which must have occurred by participation of the C-2-acetoxy-group in the opening of the episulphonium ion, as in (192). A similar reaction occurred in the galactose series. Acetolysis of the *O*-glycoside analogue of (188) gave only unchanged starting material. The participation of the thiomethyl group must have involved reaction *via* a normally unstable conformation of the ring.[284]

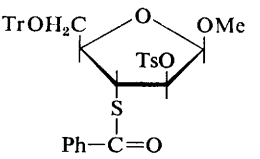
The competitive participating powers of the sulphur and oxygen atoms of the *S*-benzoyl group in the displacement of the toluene-*p*-sulphonyloxy-group in system (193) have been examined using the isomers (194) and



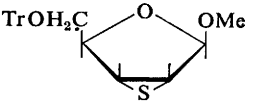
(193)



(194)



(195)

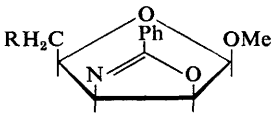


(196)

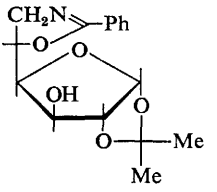
[284] E. V. E. Roberts, J. C. P. Schwarz, and C. A. NcNab, *Carbohydrate Res.*, 1968, **7**, 311.

(195). Treatment of each with sodium benzoate in DMF gave the episulphide (196) in good yield, indicating that sulphur participation through a three-membered ring occurred in preference to oxygen participation *via* a five-membered ring.[285]

(b) *Nitrogen functions.* Two papers have appeared on the synthesis of epimino-sugar derivatives which involved the participation of a neighbouring nitrogen function in the displacement of a sulphonyloxy-group (see p. 104). Participation of a benzamido-group in sulphonyloxy-displacement has led to the preparation of the oxazolines (197) and (198),[286] and (199).[287]



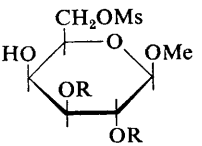
(197) R = NHBz
(198) R = OH



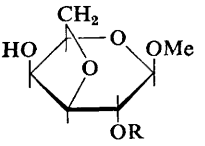
(199)

(c) *Oxygen functions.* A considerable amount of interesting work has been reported in this area, particularly by Brimacombe and his colleagues.

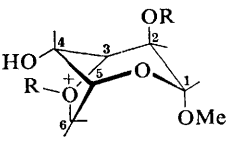
Full details have now appeared on the work on benzyloxy-participation reported briefly last year (Vol. 1, p. 76). Solvolysis of methyl 2,3-di-*O*-benzyl-6-*O*-methanesulphonyl-β-D-galactopyranoside (200) in aqueous methanol or DMF gave methyl 3,6-anhydro-2-*O*-benzyl-β-D-galactopyranoside (201). Experiments with model compounds showed that



(200)

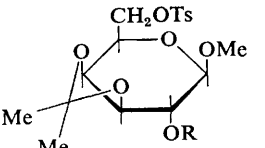


(201)



(202)

R = CH_2Ph



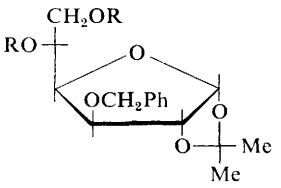
(203)

anchimeric assistance had occurred, postulated as *via* an ion, such as (202).[191] The same anhydro-sugar (201) was obtained by acid hydrolysis

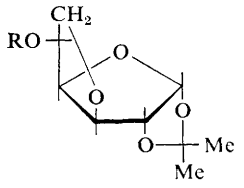
[285] K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, 1968, **33**, 3727.
[286] J. Hildesheim, E. Walczak, and S. D. Gero, *Compt. rend.*, 1968, **267**, *C*, 980.
[287] W. Meyer zu Reckendorf and N. Wassiliadou-Micheli, *Chem. Ber.*, 1968, **101**, 2294.

of the acetal group in methyl 2-*O*-benzyl-3,4-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-β-D-galactopyranoside (203) and showed that displacement of the sulphonyloxy-group had involved assistance from the C-3-hydroxy-group.[191]

The study of this type of participation has been extended to the 5,6-di-*O*-sulphonyl derivatives of 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose (204), which on treatment with sodium acetate in 50% aqueous methanol gave the appropriate 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-sulphonyl-α-D-glucofuranose (205). Under identical conditions the *allo*-analogue of (204) was virtually unchanged.[288] These results show that the conversion of (204) must have involved participation of the C-3-benzyloxy-group, as in (206).

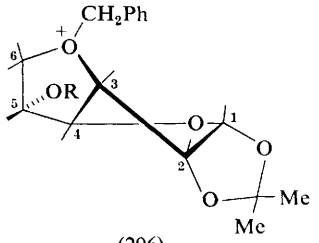


(204)



(205)

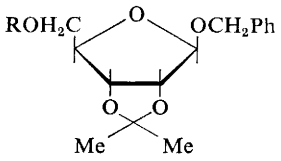
R = Sulphonyl



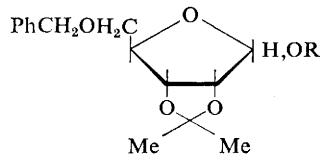
(206)

A preliminary report has been given of the solvolysis of benzyl 5-*O*-*p*-bromobenzenesulphonyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (207) in the same medium as above, which gave a mixture in which the parent alcohol (208) and 5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribofuranose (210) were the major products. Also formed in small amounts were benzyl 2,3-*O*-isopropylidene-5-*O*-methyl-β-D-ribofuranoside (209) and the methyl glycoside (211) of (210);[289] 1,5-anhydro-2,3-*O*-isopropylidene-β-D-ribofuranose was shown to be absent. Products (210) and (211) were presumed to have arisen *via* the oxonium ion intermediate (212), which opened at C-1 to give the ion (213), whilst (208) and (209) arose by direct displacement of the sulphonyloxy-group.

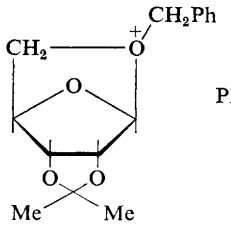
[288] J. S. Brimacombe and O. A. Ching, *Carbohydrate Res.*, 1968, **8**, 82.
[289] J. S. Brimacombe and O. A. Ching, *Carbohydrate Res.*, 1968, **8**, 374.



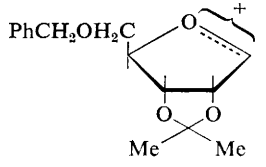
(207) R = Br·C_6H_4·SO_2
(208) R = H
(209) R = Me



(210) R = H
(211) R = Me

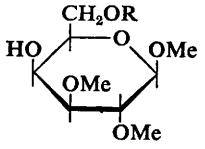


(212)

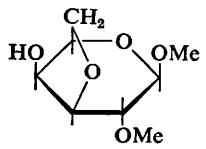


(213)

Another preliminary report from Brimacombe's group, has described participation by a methoxy-group, not attached to C-1. Solvolysis of methyl 6-*O*-methanesulphonyl-2,3-di-*O*-methyl-β-D-galactopyranoside (214) [the methoxy-analogue of (200) described above] with sodium acetate in aqueous methanol gave a mixture of methyl 3,6-anhydro-2-*O*-methyl-β-D-galactopyranoside (217) (32%), methyl 2,3,6-tri-*O*-methyl-β-D-galactopyranoside (215) (18%), and the corresponding 2,3-di-*O*-methyl ether (216)



(214) R = Ms
(215) R = Me
(216) R = H



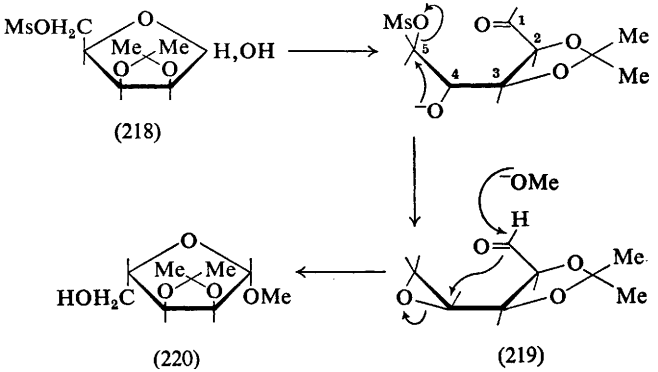
(217)

(>26%).[289a] It was presumed that (217) was formed *via* an oxonium ion analogous to (202), and that (215) and (216) were formed by direct attack, or attack on the oxonium ion at C-6. No products that would have resulted from attack on the intermediate oxonium ion at C-3 were isolated, and models showed that such an attack would be severely sterically hindered.

Brimacombe's group have also studied further the reaction first observed by Levene (*J. Biol. Chem.*, 1936, **116**, 169) in which he obtained methyl

[289a] J. S. Brimacombe and O. A. Ching, *Chem. Comm.*, 1968, 781.

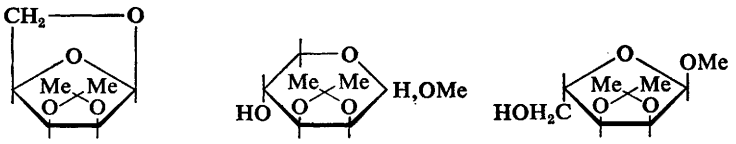
6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside from the action of methanolic sodium methoxide on 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-rhamnofuranose. It has now been shown [190] that treatment of 2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-lyxofuranose (218) under the same conditions gave methyl 2,3-*O*-isopropylidene-β-L-ribofuranose (220) identified by comparison with its known enantiomer. The reaction was postulated as proceeding by epoxide formation followed by aldehyde group participation as shown in Scheme 17. Minor products isolated in



(218) (219) (220)

Scheme 17

this reaction were identified as 1,5-anhydro-2,3-*O*-isopropylidene-β-D-lyxofuranose (221), methyl 2,3-*O*-isopropylidene-α-D-lyxopyranoside (222), methyl 2,3-*O*-isopropylidene-α-L-ribofuranoside (224), and possibly methyl



(221) (222) α-Anomer (223) β-Anomer (224)

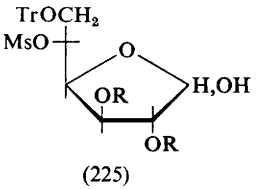
2,3-*O*-isopropylidene-β-L-lyxopyranoside (223). These products were believed to arise from the intermediate (219) by attack at the more hindered side of the carbonyl group and, additionally, opening of the epoxide ring at C-5.[290] The anhydro-sugar (221) presumably arose from (218) by attack of the C-1 hydroxy-group on the methanesulphonyloxy-group. Re-investigation of Levene's original system (*ibid.*) showed that the α-*allo*-anomer was a minor product.

This type of reaction has been further extended to 2,3:6,7-di-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-D-*glycero*-D-*gulo*-heptose, which with

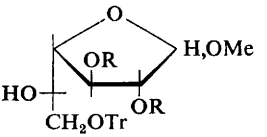
[290] J. S. Brimacombe and F. Hunedy, *J. Chem. Soc.* (*C*), 1968, 2701.

methanolic sodium methoxide gave two major products: methyl 2,3:6,7-di-*O*-isopropylidene-β-D-*glycero*-L-*talo*-heptofuranoside and 1,4-anhydro-2,3:6,7-di-*O*-isopropylidene-α-D-*glycero*-D-*allo*-heptopyranose, which must have arisen from a 4-*O*-sulphonyl compound.[291] Since esterification of the starting compound was believed to give only the 5-ester, the possibility of sulphonyl ester migration was discussed.

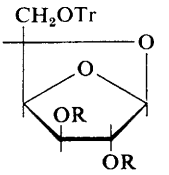
The same type of reaction was also involved in the interconversion of the D-glucofuranose configuration to the L-altrofuranose, full details of which have now been given. 2,3-Di-*O*-benzyl-5-*O*-methanesulphonyl-6-*O*-trityl-D-glucofuranose (225) (prepared from 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose by standard sequences *via* methyl 2,3-di-*O*-benzyl-α/β-D-glucofuranoside 5,6-carbonate) on treatment with methanolic sodium



(225)



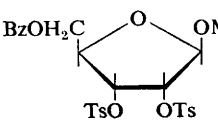
(226)
R = CH_2Ph



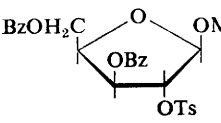
(227)

methoxide gave[292] an anomeric mixture of methyl 2,3-di-*O*-benzyl-6-*O*-trityl-L-altrofuranosides (226), by a mechanism analogous to that shown in Scheme 17. A minor product isolated was believed to be (227).

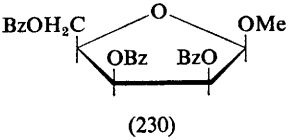
Treatment of methyl 5-*O*-benzoyl-2,3-di-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside (228) with sodium benzoate in DMF gave three products, (229), (230), and (231) in yields of 17, 7, and 30% respectively; benzoylation



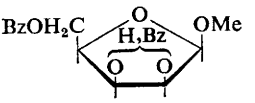
(228)



(229)



(230)



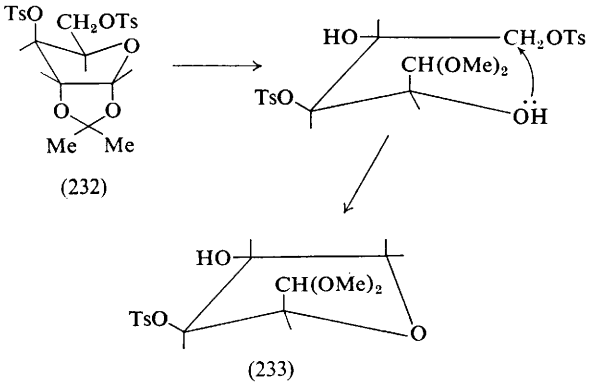
(231)

of (231) gave (230). It was considered that the *xylo*-product (229) arose by a selective S_N2 displacement at C-3 and that the *lyxo*-compound (230)

[291] J. S. Brimacombe and L. C. N. Tucker, *J. Chem. Soc.* (*C*), 1968, 562.
[292] H. Saeki, T. Iwashige, and E. Ohki, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1040.

probably also arose by direct displacements; the formation of the di-*O*-benzoyl derivative (231) occurred by participation of a benzoyloxy-group, presumably at C-3, in the displacement of the C-2-sulphonyloxy-group. This idea received support when it was shown that (229) gave (231) on treatment with sodium fluoride in DMF. De-esterification of (230) gave the hitherto unknown methyl-β-D-lyxofuranoside, which on acid hydrolysis gave the free sugar.[293]

Hydroxy-group participation has been invoked in the reaction of 1,2-*O*-isopropylidene-3,5-di-*O*-toluene-*p*-sulphonyl-D-xylofuranose (232),

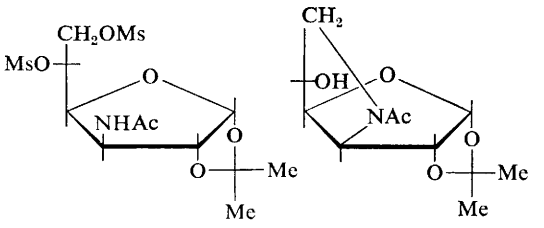


(232) (233)

Scheme 18

which with methanolic hydrogen chloride gave 2,5-anhydro-3-*O*-toluene-*p*-sulphonyl-D-xylose dimethyl acetal (233); the pathway shown in Scheme 18 was proposed.[294]

The solvolysis of 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-5,6-di-*O*-methanesulphonyl-α-D-glucofuranose (234) was undertaken to see if participation of the acetamido-group would occur (*cf.* ref. 288). The



(234) (235)

product was shown to have structure (235). A mechanism was proposed, which involved *O*- and then *N*-participation.[295] The L-*ido*-configuration of

[293] J. Hildesheim, J. Cleophax, and S. D. Gero, *Compt. rend.*, 1968, **267**, *C*, 1070.
[294] J. Defaye and J. Hildesheim, *Tetrahedron Letters*, 1968, 313.
[295] J. S. Brimacombe and J. G. H. Bryan, *Carbohydrate Res.*, 1968, **6**, 423.

(235) was further confirmed by the resistance of its 5-*O*-methanesulphonyl derivative to displacement by azide or benzoate ions, suggesting that it had an *exo*-configuration.

Nitrates

Thymidine 5′-nitrate has been prepared by a new route involving the use of dialkylthiophosphorochloridates and silver nitrate in a reaction which was believed to involve the intermediate (236). Nucleophilic attack of an

$$(EtO)_2\overset{\overset{S}{\|}}{P}ONO_2$$

(236)

alcohol at nitrogen then gave the nitrate. Acetates may be synthesised in the same way.[296]

Borates and Boronates

Two papers have appeared on the preparation of non-specific polyol borates. Polymeric esters of D-mannitol formed from different ratios of reactants were examined,[297] and in related work[298] crystalline polymers were obtained from calcium and strontium galactosaccharates. Related esters of xylitol and anhydroxylitol were also reported.[299]

The 2′,3′-phenylboronate of adenosine has been used in the preparation of 5′-esters, phorphorylation being accomplished with 75% efficiency with phosphoromorpholidic dichloride and diphenyl phosphate.[300] Similarly, the 2′,3′-phenylboronate-5′-toluene-*p*-sulphonate was obtained,[301] the reactions of which were studied.

[296] I. Schwandt, H. Teichmann, G. Hilgetag, G. Kowollik, and P. Langen, *Z. Chem.*, 1968, **8**, 176.
[297] E. Svarcs, V. Grundsteins, and A. Ievins, *Latv. P.S.R. Zinat. Akad. Vestis, Kim. Ser.*, 1967, 154.
[298] E. Svarcs, V. Grundsteins, and A. Ievins, *Latv. P.S.R., Zinat. Akad. Vestis, Kim. Ser.*, 1967, 409.
[299] R. I. Zamanskaya, V. N. Balakhontseva, and A. N. Kholmyanskaya, *Zhur. priklad. Khim.*, 1968, **41**, 1122.
[300] A. M. Yurkevich, I. I. Kolodkina, G. S. Evdokimova, E. T. Bazhanova, and N. A. Preobrazhenskii, *Khim. Org. Soedin. Forsora, Akad. Nauk S.S.S.R. Otdel. Obshchei. Tekh. Khim.*, 1967, 215.
[301] A. A. Amagaeva, A. M. Yurkevich, I. P. Rudakova, L. V. Khristenko, I. M. Custanovich, and N. A. Preobrazhenskii, *Khim. prirod. Soedinenii*, 1968, 304.

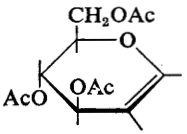
7
Halogenated Sugars

The oxo-reaction as applied to halogenated sugars has been reviewed.[301a]

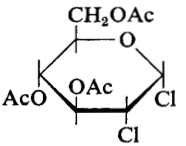
Glycosyl Halides

Optical rotations of acyl glycosyl halides have been examined in detail (see p. 203).

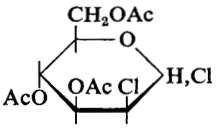
The addition of chlorine to tri-*O*-acetyl-D-glucal (237) in carbon tetrachloride has been further examined and shown to give mainly the 2-chloro-2-deoxy-α-D-glucopyranosyl chloride (238) (63%), isolated together with a second product (13%) previously assigned the α-*manno*-structure (239) but now shown to be the β-*manno*-product (240). Additionally, however, the



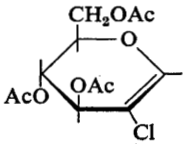
(237)



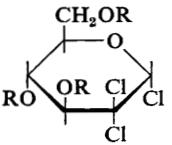
(238)



(239) α-Anomer
(240) β-Anomer



(241)



(242) R = Ac
(243) R = H

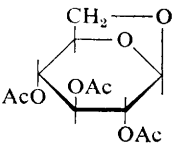
α-*manno*-adduct was obtained directly (5%), and also by anomerisation of (240). Reaction of (237) with *N*-chlorosuccinimide gave all four adducts in the ratio α-*gluco*, 15·7 : α-*manno*, 1·4 : β-*manno*, 4·4 : β-*gluco*, 10·7. The mechanisms of the addition reactions were discussed.[302] Other workers confirmed the main findings of this study and also prepared (241) by treatment of (238) with diethylamine. Compound (241) reacted with chlorine to give crystalline (242) which yielded a stable deacetylated product (243), believed to be the first example of a stable unsubstituted

[301a] A. Rosenthal, *Adv. Carbohydrate Chem.*, 1968, **23**, 60.
[302] K. Igarashi, T. Honna, and T. Imagawa, *Tetrahedron Letters*, 1968, 755.

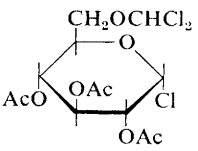
glycosyl chloride. The stability of (243), further exhibited by its failure to react with methanol, was attributed to the inductive influences of the C-2 chlorine atoms which retard ionisation at the anomeric centre.[303]

A substituted xylosyl chloride was prepared on treatment of D-xylose with sulphuryl chloride (see p. 59); the complex formed in the reaction between penta-*O*-acetyl-β-D-glucopyranose and titanium tetrachloride has been shown to be a 1 : 1 complex of β-acetochloroglucose and acetoxytitanium trichloride.[304]

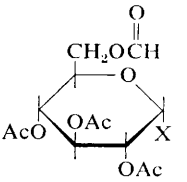
2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (244) reacted with an excess of dichloromethyl methyl ether to give (245) and with 1·2 mole of the reagent to give (246). Both (245) and (246) were converted to the



(244)



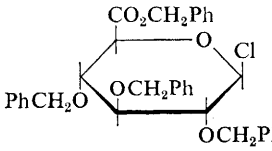
(245)



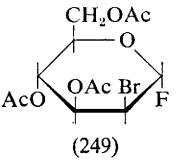
(246) X = Cl
(247) X = Br

β-acetate derived from (246). The limited-scale reaction with dibromomethyl methyl ether gave the glycosyl bromide (247) which, like (246), gave alkyl glucosides on treatment with alcohols in the presence of silver carbonate. Compound (247) was converted to the chloro-analogue on treatment with titanium tetrachloride.[305]

Treatment of benzyl 2,3,4-tri-*O*-benzyl-D-glucuronate with thionyl chloride at 0° gave a product shown to be (248) which with benzyl alcohol afforded the α-benzyl glycoside. Compound (248) and its α-anomer were investigated by n.m.r. spectroscopy (see p. 194) and the former was shown to adopt a conformation other than the expected *C*1.[306]



(248)



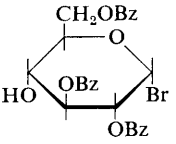
(249)

Glycosyl fluorides are important intermediates formed in the reaction of esters with hydrogen fluoride.[206, 228] A specific fluoride produced by the reaction of *N*-bromosuccinimide and anhydrous hydrogen fluoride with

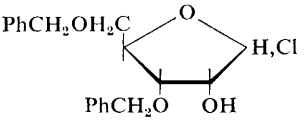
[303] P. R. Bradley and E. Buncel, *Canad. J. Chem.*, 1968, **46**, 3001.
[304] Z. Csürös, Gy. Deák, L. Fenichel, S. Holly, and J. Pálinkás, *Acta Chim. Acad. Sci. Hung.*, 1968, **56**, 325.
[305] R. Bognár, I. Farkas, M. Menyhárt, H. Gross, and H. Paulsen, *Carbohydrate Res.*, 1968, **6**, 404.
[306] N. Pravdić and D. Keglevic, *Carbohydrate Res.*, 1968, **7**, 167.

tri-*O*-acetyl-D-glucal has been shown by crystallographic analysis to be (249) and not the β-anomer as was previously claimed.[307] Alternatively, this compound was the main product (70%) of addition of 'BrF' (bromine and silver fluoride), and the α-*gluco*- and β-*gluco*-compounds were also produced in 9 and 21% yield, respectively. The products were examined by n.m.r. methods (see p. 197); silver fluoride–iodine yielded the 2-iodo-2-deoxy-analogues in 60, 6, and 43% yield.[308]

2,3,6-Tri-*O*-benzoyl-α-D-glucopyranosyl bromide (250) has been synthesised from the 1,2,3,6-tetrabenzoate, and its structure established by its conversion into the known methyl β-glucoside. Attempts at self-condensation of (250) to give β-1,4-cellodextrins were unsuccessful, presumably because of the steric inaccessibility of the hydroxy-group.[309]



(250)



(251)

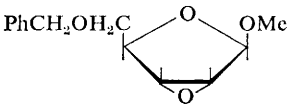
A method of preparing ribofuranosyl halides involved the use of 3,5-di-*O*-benzyl-2,4-*O*-benzylidene-D-ribose obtained from the known 2,4-*O*-benzylidene-D-ribose dipropyl dithioacetal. 3,5-Di-*O*-benzyl-D-ribofuranose was thus synthesised and from it the corresponding glycosyl chloride (251) which was utilised in nucleoside condensations.[310]

Other Halogenated Derivatives

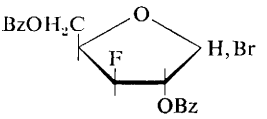
Newer methods for the synthesis of deoxyhalogeno-sugars have been reviewed.[311]

Most new results in this series relate to fluoro-derivatives. A synthesis of 3-deoxy-3-fluoro-D-xylose from methyl 2,3-anhydro-5-*O*-benzyl-β-D-ribofuranoside (252) with potassium hydrogen difloride has been described. The initial product was debenzylated, benzoylated, and converted to the bromides (253) which with benzamidopurine afforded means of obtaining (254). A similar set of reactions with methyl 5-*O*-benzyl-3-deoxy-3-fluoro-α-D-arabinofuranoside gave (255).[312] The synthesis of various related halogenated nucleosides from anhydronucleosides has been discussed, and n.m.r. studies were carried out on several glycosyluridines bearing halogen atoms at C-2′ and -3′ (see p. 196).[313] A 3′-chloro-3′-deoxy-nucleoside is reported on p. 126.

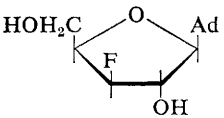
[307] J. C. Campbell, R. A. Dwek, P. W. Kent, and C. K. Prout, *Chem. Comm.*, 1968, 34.
[308] L. D. Hall and J. F. Manville, *Chem. Comm.*, 1968, 35.
[309] W. W. Wadsworth, L. R. Schroeder, and J. W. Green, *J. Chem. Soc.* (*C*), 1968, 1008.
[310] M. Haga, R. K. Ness, and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 1810.
[311] S. Hanessian, *Adv. Chem. Ser. No.* 74, 1968, 159.
[312] J. A. Wright and N. F. Taylor, *Carbohydrate Res.*, 1968, **6**, 347.
[313] R. J. Cushley, J. F. Codington, and J. J. Fox, *Canad. J. Chem.*, 1968, **46**, 1131.



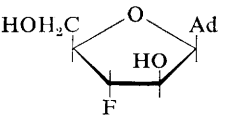
(252)



(253)



(254)



(255)

The synthesis of 3-deoxy-3-fluoro-D-galactose has already been described.[273] A direct means of introducing fluorine was observed during the reaction of 1,2:3,5-di-*O*-methylene-α-D-glucofuranose with (256) which

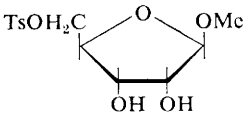
$$ClFHC \cdot CF_2NEt_2$$

(256)

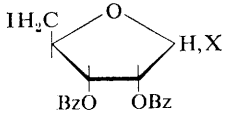
gave the 6-deoxy-6-fluoro-derivative which could also be prepared by nucleophilic displacement of 6-sulphonates.[214] N.m.r. features of fluorinated carbohydrates have been reported (see p. 197).

Conditions have been developed for the efficient and direct substitution of the primary hydroxy-group of hexopyranosides by chlorine using methanesulphonyl chloride in DMF. Methyl α-D-xylopyranoside was unreactive but many primary hydroxy-containing compounds underwent the displacement; 6-*O*-methanesulphonates were not intermediates. The use of methanesulphonyl fluoride did not lead to 6-deoxy-6-fluoro-glycosides, and methanesulphonic anhydride gave formyl esters. From the chloro-products, hydroxy-groups could be regenerated by way of benzoate esters (using nucleophilic displacements in DMF).[269] Other chlorinated products obtained during sulphonylations have been mentioned.[268]

Ribofuranosyl derivatives containing an iodine atom at C-5 were synthesised by way of (257) and thus compounds (258) and (259) were prepared.[314]



(257)



(258) X = OAc
(259) X = Br

[314] H. Pischel and G. Wagner, *Z. Chem.*, 1968, **8**, 178.

6-Deoxy-6-iodo-compounds can be reduced photolytically to the corresponding 6-deoxy-derivatives (see p. 127), and iodo-compounds, in general, offer a means of introducing unsaturation into sugars (p. 129).

Other halogenated compounds are referred to in Chapter 14.

8
Amino-sugars*

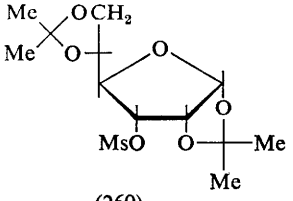
Naturally-occurring Compounds†

An amino-sugar isolated after hydrolysis of the lipopolysaccharide of *E. coli* U41/14 has been shown to be 2-amino-2,6-dideoxy-L-mannose.[315] Full details of the isolation of 3-amino-3-deoxy-D-glucose (kanosamine) from the broth of *Bacillus aminoglucosidicus* have been reported[316] (*cf.* Vol. 1, p. 87) and its biosynthesis has been studied.[317] For the first time it has been shown that *N*-acetylneuraminic acid is an important constituent of sea urchin jelly.[317a]

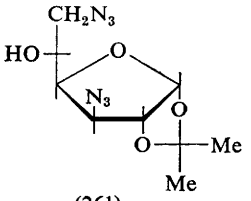
Synthesis‡

As in previous years the azido-group has retained its place as the most used precursor of the amino-group in synthesis, introduction being by either sulphonyloxy-group displacement or by epoxide ring-opening.

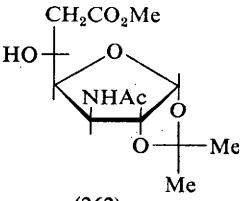
3-Acetamido-3-deoxy-D-glucose has been prepared (*cf.* Vol. 1, p. 90) by the ready displacement of the sulphonyloxy-group from 1,2:5,6-di-*O*-isopropylidene-3-*O*-methanesulphonyl-α-D-allofuranose (260). The intermediate 3-azido-3-deoxy-D-glucofuranose derivative was partially hydrolysed and converted by standard methods into the diazido-derivative (261)



(260)



(261)



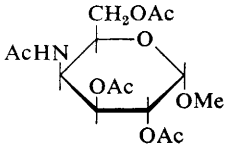
(262)

[315] B. Jann and K. Jann, *European J. Biochem.*, 1968, **5**, 173.
[316] S. Umezawa, K. Umino, S. Shibahara, M. Hamada, and S. Omoto, *J. Antibiotics (Japan), Ser. A*, 1967, **20**, 355.
[317] S. Umezawa, S. Shibahara, S. Omoto, T. Takeuchi, and H. Umezawa, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 485.
[317a] J. Immers, *Acta Chem. Scand.*, 1968, **22**, 2046.

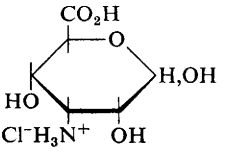
* See also Chapter 12.
† See also Chapter 20.
‡ See also Chapter 6.

(from which 3,6-diacetamido-3,6-dideoxy-D-glucose was prepared) and into the uronic acid derivative (262).[317b]

A preliminary report has appeared on the synthesis of methyl 2,3,6-tri-*O*-acetyl-4-acetamido-4-deoxy-α-D-galactopyranoside (263) by standard sequences from methyl 2,3-di-*O*-benzyl-4-*O*-methanesulphonyl-6-*O*-trityl-α-D-glucopyranoside (*via* the 4-azido-4-deoxy-galactoside) and also from methyl 2,3-di-*O*-benzyl-4,6-di-*O*-methanesulphonyl-α-D-glucopyranoside, in which the sulphonyloxy-group on C-6 was selectively displaced by benzoate ion.[318] The configuration of (263) was established by n.m.r. methods. A product from the degradation of the nucleoside antibiotic gougerotin has previously been assigned structure (263), but the product now synthesised had different physical constants, throwing doubt on the original assignment (*cf.* p. 179).

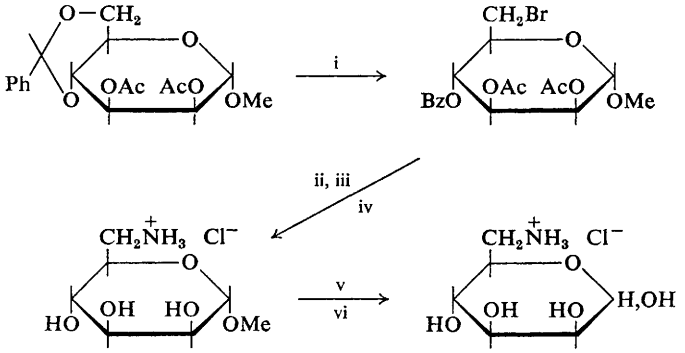


(263)



(264)

Three methods have been described for the synthesis of 3-amino-3-deoxy-D-alluronic acid (264), all starting from 1,2-*O*-isopropylidene furanose compounds.[319] One involved displacement of a sulphonyloxy-group by azide ion, the other two used a 3-keto-derivative in the form of its oxime or



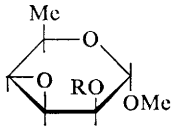
Reagents: i, NBS–CCl_4; ii, NaN_3–DMF; iii, H_2–Ni; iv, MeO^-; v, Ac_2O–py; vi, aq. HCl

Scheme 19

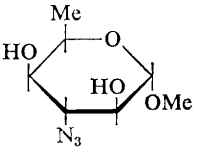
[317b] W. Meyer zu Reckendorf, *Chem. Ber.*, 1968, **101**, 3802.
[318] F. W. Lichtenthaler and P. Heidel, *Angew. Chem. Internat. Edn.*, 1968, **7**, 458.
[319] A. Tsuji, T. Kinoshita, and M. Maeda, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 539.

phenylhydrazone. A useful synthesis of 6-amino-6-deoxy-D-mannose has been reported, as shown in Scheme 19.[320] The displacement of the sulphonyloxy-group in 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-gulofuranose with azide ion has been used in a new route to 3-acetamido-3-deoxy-β-D-galactopyranose.[274]

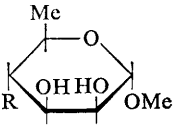
In an effort to find routes to the biologically important 4-amino-4,6-dideoxy-sugars, the ring-opening of methyl 3,4-anhydro-6-deoxy-α-D-talopyranoside (265) and its 2-*O*-benzoyl derivative (266) with azide ion has been investigated.[321] As expected, (265) gave predominantly the product resulting from ring-opening at C-3, namely (267) (45%), together with the 4-azido-4-deoxy-derivative (268) (17%). Opening of (266) gave a more



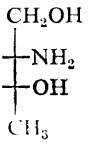
(265) R = H
(266) R = Bz



(267)



(268) R = N_3
(269) R = NH_2



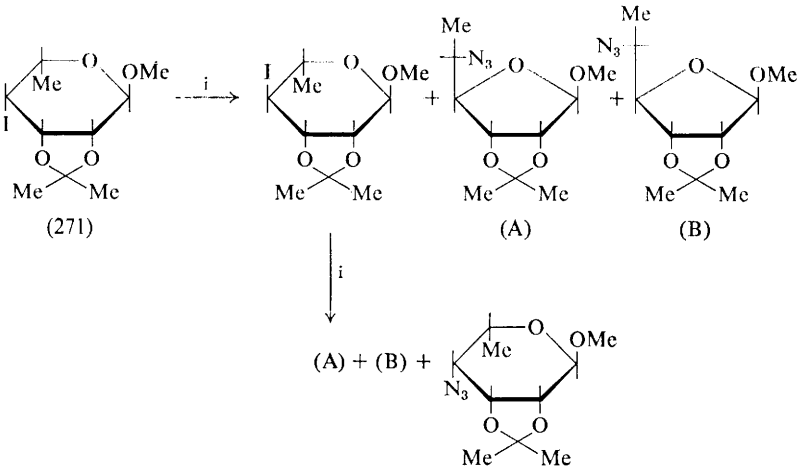
(270)

complex reaction with even less opening at C-4. The configuration of (268) was established by the sequence: acid hydrolysis, borohydride reduction, periodate oxidation, borohydride reduction, to give the known 2-amino-2,4-dideoxy-D-erythritol (270) as its hydrogen oxalate salt. Reduction of (268) gave methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (269), identical with methyl perosaminide, obtained from the methanolysis of the antibiotic perimycin.

The transformations shown in Scheme 20 were found to occur on treatment of the iodo-compound (271) with sodium azide in DMF.[322] [This reaction should be compared with the similar reactions described on the corresponding methanesulphonyloxy-derivative [282] (*cf.* Vol. 1, p. 79).] Reduction of the products and acetylation gave new methods for obtaining 4- and 5-acetamido-derivatives.

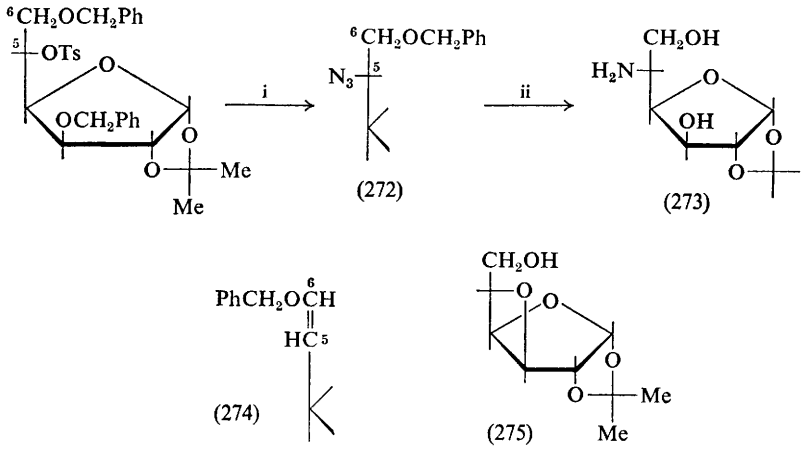
5-Amino-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose has been synthesised by the route shown in Scheme 21; in addition to (272), the unsaturated sugar (274) was obtained as a minor product from the displacement reaction, together with a third compound believed to be an isomeric olefin. Alternatively, compound (273) was obtained by opening of the oxetan ring in (275) with azide ion, followed by reduction;[323] improvements in the preparation of (275) were described.

[320] D. Horton and A. E. Luetow, *Carbohydrate Res.*, 1968, **7**, 101.
[321] C. L. Stevens, S. K. Gupta, R. P. Glinski, K. G. Taylor, P. Blumbergs, C. P. Schaffner, and C.-H. Lee, *Carbohydrate Res.*, 1968, **7**, 502.
[322] A. I. Usov, K. S. Adamyants, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2546.
[323] U. G. Nayak and R. L. Whistler, *J. Org. Chem.*, 1968, **33**, 3582.



Reagents: i, NaN_3–DMF

Scheme 20



Reagents: i, NaN_3–DMF; ii, NH_3(liq.)–Na

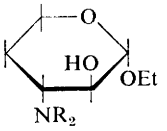
Scheme 21

Opening of an epoxide ring with amino-compounds has also been used in the synthesis of compounds (276)[324] and (277).[325]

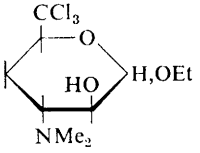
The dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glycopyranosyl chlorides, obtained in excellent yield from the addition of nitrosyl chloride

[324] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1968, **38**, 85.

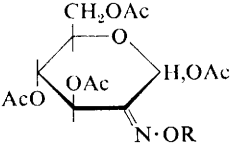
[325] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1968, **38**, 427.



(276)



(277)

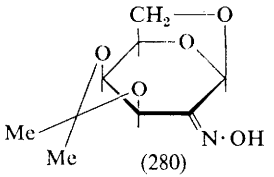


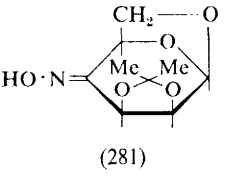
(278) R = H
(279) R = Ac

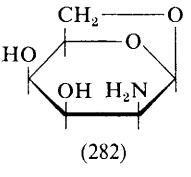
to tri-*O*-acetyl-glycals, have now been shown to react readily with alcohols in DMF at room temperature to give tri-*O*-acetyl-2-oximino-α-D-hexopyranosides in excellent yield.[80] Hydrogenation of the compound with the D-*arabino*-configuration over palladium catalysts in the presence of hydrochloric acid gave alkyl 2-amino-2-deoxy-α-D-glucosides and -mannosides in high yield.[326] The stereoselectivity of the reduction depended on the aglycone and on whether or not the hydroxy-group on the oximino-group was acylated. Appreciable amounts of both isomers were, however, usually obtained; reduction of methyl 6-*O*-(2-oximino-α-D-*arabino*-hexopyranosyl)-β-D-glucopyranoside gave a 1 : 1 mixture of the two aminodisaccharide derivatives.[326] Acetolysis of the dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-hexopyranosyl chlorides gave, in the D-*arabino*-hexose case, compound (278), which on reduction with a zinc–copper couple in acetic acid furnished per-acetylated 2-amino-2-deoxy-D-glucose in good yield.[327] Alternatively, treatment of the glycosyl chloride with a base gave, in the same example, compound (279), which on catalytic hydrogenation in acetic anhydride–acetic acid over palladium gave acetylated 2-amino-2-deoxy-D-mannose, again in good yield. 2-Amino-2-deoxy derivatives of D-galactose and D-talose were prepared analogously from 3,4,6-tri-*O*-acetyl-D-galactal.[327]

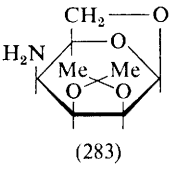
The reduction of oximes has also been used in a number of other preparations of amino-sugars, including a new route to 3-amino-3-deoxy-D-ribose.[328] Hydrogenation of the oximes of 1,6-anhydro-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-2-ulose and of 1,6-anyydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose, (280) and (281), respectively, over a platinum catalyst in hydrochloric acid solution gave the aminodeoxy-products (as their hydrochlorides) which resulted from attack of the oxime group from the least hindered face of the molecule, *i.e.* (282) and (283); concomitant loss of the acetal group also occurred.[329] Hydrolysis of (282) gave the amino-sugar hydrochloride as a mixture of α- and β-pyranose anomers in a 1 : 2 ratio; further hydrolysis of (283) gave only tarry products, presumably due to decomposition of the free 4-amino-sugar *via* pyrolle intermediates. 4,6-Dideoxy-4-dimethylamino-D-talopyranose hydrochloride

[326] R. U. Lemieux and S. W. Gunner, *Canad. J. Chem.*, 1968, **46**, 397.
[327] R. U. Lemieux and T. L. Nagabhushan, *Canad. J. Chem.*, 1968, **46**, 401.
[328] W. Sowa, *Canad. J. Chem.*, 1968, **46**, 1586.
[329] A. K. Chatterjee, D. Horton, J. S. Jewell, and K. D. Phillips, *Carbohydrate Res.*, 1968, **7**, 173.


(280)


(281)

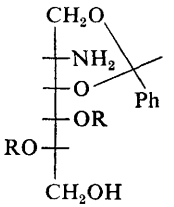

(282)

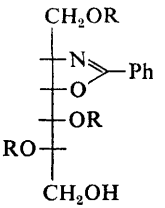

(283)

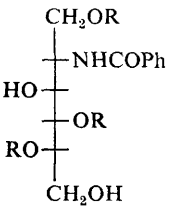
has been prepared from methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-manno-pyranoside using oxidation to the 4-ulose and reduction of the oxime as the key step.[330]

3′-Amino-3′-deoxy-pyranosyl nucleotides have been prepared *via* the nitromethane cyclisation reaction (see p. 101). 2-Amino-2-deoxy-D-talose and -D-galactose have been prepared from the corresponding 2-acetamido-1,2-dideoxy-1-nitrohexitols;[331] 2-amino-2-deoxy-D-allose and -altrose have been similarly obtained.[332] Two epimeric pairs of 2-acetamido-1,2-dideoxy-1-nitroheptitols with the D-*glycero*-D-*talo*- and -D-*galacto*-, and the D-*glycero*-L-*manno*- and -L-*gluco*-configurations have been described.[333]

Derivatives (284) and (285) of 2-amino-2-deoxy-L-talitol have been prepared as possible intermediates in the stereospecific synthesis of sphingosine. The former derivative (284) was prepared from 4,5-di-*O*-benzyl-1,3-*O*-benzylidene-L-galactitol by standard methods; the synthesis


(284)


(285)

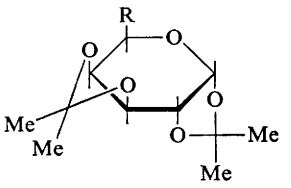

(286)

R = CH_2Ph

of the compound (285) was from an anomeric mixture of methyl 2,3,6-tri-*O*-benzyl-α-D-glucofuranoside, which was converted into 2-benzamido-1,4,5-tri-*O*-benzyl-2-deoxy-L-iditol (286) and thence to (285).[334]

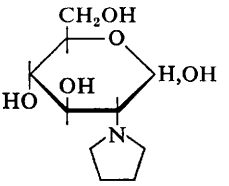
[330] C. L. Stevens, R. P. Glinski, and K. G. Taylor, *J. Org. Chem.*, 1968, **33**, 1586.
[331] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1968, **46**, 2481.
[332] M. B. Perry and J. Furdová, *Canad. J. Chem.*, 1968, **46**, 2859.
[333] C. Satoh and A. Kiyomoto, *Carbohydrate Res.*, 1968, **7**, 138.
[334] R. Gigg and C. D. Warren, *J. Chem. Soc.* (*C*), 1968, 2661.

6-Amino-6-deoxy-D-galactose and some of its derivatives (287) have been synthesised from 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-dialdo-1,5-



(287) R = CH(CN)—NHCH$_2$Ph or CH(CN)NH$_2$ or CH(CN)OH

(288) R = CHO



(289)

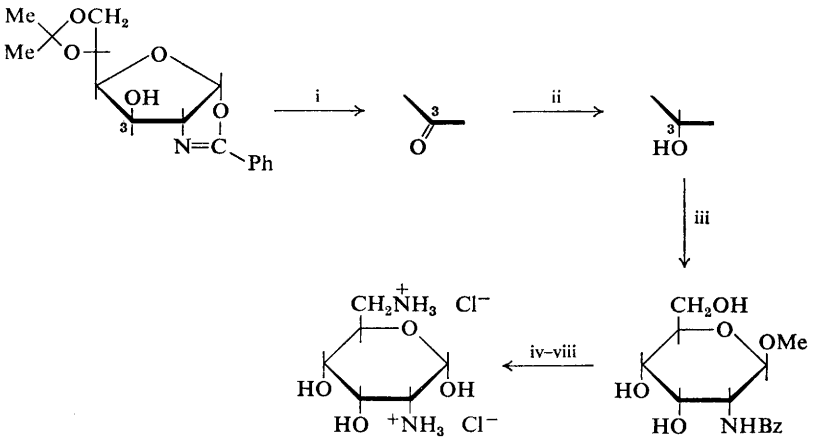
pyranose (288), to investigate their biological activity relative to lincomycin.[335]

Reaction of D-fructose with pyrollidine gave the 2-deoxy-2-pyrollidine-derivative (289) in 15% yield. The mass spectrum of the tetra-*O*-acetyl derivative was compared with that of other amino-sugar derivatives.[336]

All of the methods discussed so far in this sub-section have involved the introduction of an amino-group into a suitable precursor. Many other syntheses have involved the modification of molecules already bearing an amino-group, generally blocked in some way. The synthesis of methyl 2-acetamido-2-deoxy-α-D-galactopyranoside and methyl 2-acetamido-2,4,6-trideoxy-α-D-*ribo*-hexopyranoside have been described earlier in this Report.[270] A new synthesis of 2,6-diamino-2,6-dideoxy-D-allose has been achieved by the route shown in Scheme 22.[337]

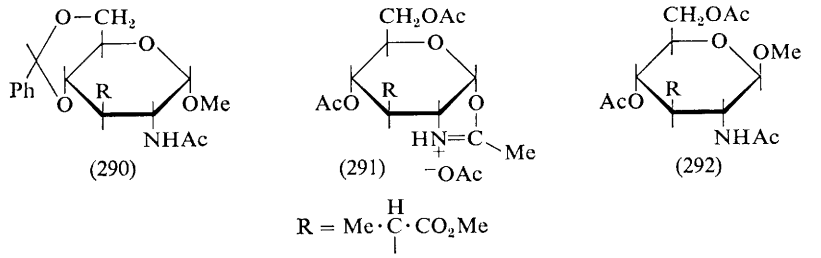
To investigate the influence of the aglycone on the condensation of (±)-2-chloropropionic acid with 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosides, the methyl, benzyl, and *p*-nitrophenyl glycosides have been studied,[338] as well as the corresponding methyl α-glucoside. The yields of the 3-*O*-(D-1-carboxyethyl) derivatives were 46, 68, 25, and 64%, respectively. Only from the α-glucoside was any significant amount of the L-1-carboxyethyl isomer (isomuramic acid derivative) isolated. Treatment of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (290) under acetolysis conditions gave the cyclic derivative (291), which on further treatment with hydrogen bromide in acetic acid, and then with methanol in the presence of silver oxide, gave the β-anomer (292) also obtained by the direct reaction and appropriate subsequent reactions.[338]

[335] H. Saeki, T. Iwashige, E. Ohki, K. Furuya, and M. Shirasaka, *Ann. Sankyo Res. Lab.*, 1967, **19**, 137 (*Chem. Abs.*, 1968, **68**, 96075f).
[336] K. Heyns, K.-W. Pflughaupt, and D. Müller, *Chem. Ber.*, 1968, **101**, 2807.
[337] W. Meyer zu Reckendorf and J. Feldkamp, *Chem. Ber.*, 1968, **101**, 2289.
[338] R. W. Jeanloz, E. Walker, and P. Sinay, *Carbohydrate Res.*, 1968, **6**, 184.



Reagents: i, Ac_2O–DMSO; ii, $NaBH_4$, iii, MeOH–HCl; iv, TsCl (1 mol.)–py; v, NaN_3–DMF; vi, Pd–H_2; vii, Bz_2O–MeOH; viii, aq. HCl

Scheme 22

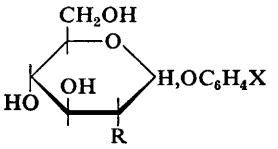


A number of papers relating to the synthesis of amino-sugar glycosides (including disaccharides) have already been mentioned [77, 81–85] and in one the dichloroacetyl group was used as an amino-blocking group.[84]

Derivatives containing isothiocyanate or bromoacetate groups have been prepared for use as potential protein alkylating agents. The compounds, prepared *via* the corresponding *p*-nitrophenyl derivatives, followed standard conversions,[339] *e.g.* (293) and (294).

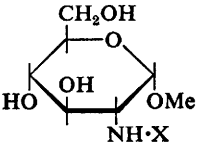
6-Amino-6-deoxy-L-idose has been synthesised by hydrolysis of the known 1,2-*O*-isopropylidene derivative (295). It proved to be unstable in the free form, reverting to the bicyclic form (296).[287] In related fashion L-idose is readily converted into its 1,6-anhydride. A series of compounds related to (295), but with the D-*gluco*-configuration, was also described, as was the L-*ido*-oxazoline (199) prepared from the corresponding 6-benzamido-6-deoxy-5-toluene-*p*-sulphonyl-D-glucose derivative.[287] D-*threo*-Pentulose (D-xylulose) reacted with cyclohexylamine to give (297), isolated

[339] D. H. Buss and I. J. Goldstein, *J. Chem. Soc.* (*C*), 1968, 1457.



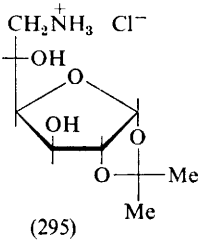
(293)

Both anomers; all combinations of X = NCS or $NHCOCH_2Br$ and R = OH or NHCOMe.

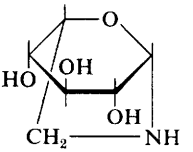


(294)

X = $COCH_2Br$ or $COC_6H_4NHCOCH_2Br$ or COC_6H_4NCS

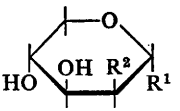


(295)

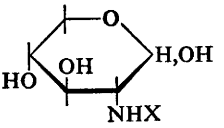


(296)

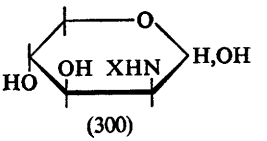
in 14% yield, which on acid hydrolysis gave the free sugar (298).[340] With β-alanine or ε-aminocaproic acid, the ketose gave a mixture of the *xylo*- and *lyxo*-compounds (299) and (300) from which the former was isolated.



(297) $R^2 = R^1 = C_6H_{11}$
(298) $R^2 = C_6H_{11}$, $R^1 = OH$



(299)



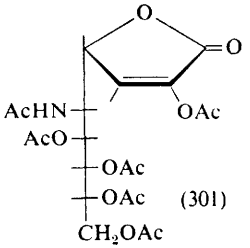
(300)

X = $(CH_2)_2CO_2H$ or $(CH_2)_5CO_2H$

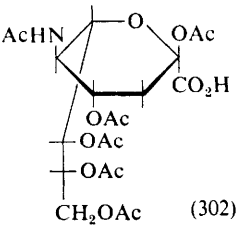
Several 2′-amino-2′-deoxy-nucleosides have been synthesised by established methods, and it was shown that the acetyl group was an effective amino-blocking group for the condensation reactions.[341]

Two papers have appeared on the synthesis of *N*-acetylneuraminic acid derivatives: one described the addition of β-D-galactosyl and -glucopyranosyl groups to the 9-position,[342] and the other showed that acetylation with acetic anhydride in pyridine gave (301) and (302) in yields of 13 and 61%, respectively.[343]

[340] K. Heyns, K.-W. Pflughaupt, and H. Paulsen, *Chem. Ber.*, 1968, **101**, 2800.
[341] M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, 1968, **33**, 4227.
[342] A. Ya. Khorlin and I. M. Privalova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 215.
[343] A. Ya. Khorlin and I. M. Privalova, *Khim. prirod. Soedin.*, 1967, **3**, 191.



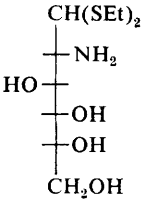
(301)



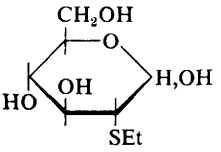
(302)

Reactions

Deamination of 2-amino-2-deoxy-D-glucose diethyl dithioacetal (303) with nitrous acid in dilute hydrochloric acid gave 2-*S*-ethyl-2-thio-D-glucose (304) as the major product, characterised by n.m.r. and by conversion into



(303)



(304)

D-*arabino*-hexulose phenylosazone and into the anomeric pyranose tetra-*O*-acetyl derivatives.[344] This result contrasts with that reported by Defaye (Vol. 1, p. 92) in which nitrous acid in aqueous acetic acid was the reagent and 2,5-anhydro-D-glucose diethyl dithioacetal was the major product. The present work [344] has shown that (304) is formed as a by-product in the latter reaction. Possible mechanisms for the reactions involving 1,2-episulphonium ions were discussed.

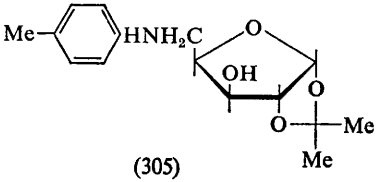
Methanolysis of 3-amino-3,6-dideoxy-1,2-isopropylidene-α-D-allofuranose gave the anomeric α- and β-pyranosides, establishing that furanose-to-pyranose interconversions occur during such reactions.[61] Methanolysis of the 5-amino-D-xylose derivative (305) gave the pyridinium derivative (306), presumably *via* the piperidinose form of the sugar.[345]

A preliminary account has been given of a method for the determination of the stereochemistry and absolute configuration at C-4 and C-5 in 4-amino-4,6-dideoxy-hexoses.[346] The 4-acetamido-4,6-dideoxy-hexoside is subjected to the sequence: periodate oxidation, borohydride reduction, acid hydrolysis (of both the glycosidic linkage and the amide group), and

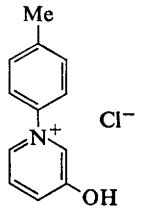
[344] A. E. El Ashmawy, D. Horton, L. G. Magbanua, and J. M. J. Tronchet, *Carbohydrate Res.*, 1968, **6**, 299.

[345] V. I. Veksler, *Zhur. obshchei Khim.*, 1968, **38**, 1649.

[346] C. L. Stevens, S. K. Gupta, R. P. Glinski, G. E. Gutowski, and C. P. Bryant, *Tetrahedron Letters*, 1968, 1817.



(305)

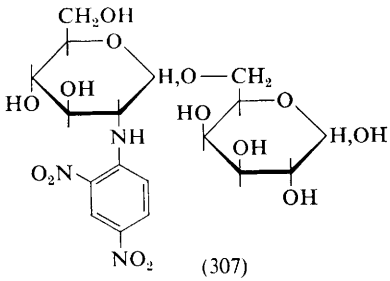


(306)

formation of the crystalline hydrogen oxalate salt, which is either the D- or L-allothreoninol salt or the D- or L-threoninol salt. Identity may be established by m.p. or mixed m.p., as well as spectroscopically and polarimetrically if sufficient material is available.

Studies on the kinetics of the acid-catalysed hydrolysis of 2-acetamido-2-deoxy-glucosides have been mentioned earlier in this Report.[107,145]

It has been shown that conversion of an alkyl 2-amino-2-deoxy-glycoside into its *N*-2,4-dinitrophenyl derivative facilitates cleavage of the glycosidic linkage under acid conditions (3N-acid at 100° for 10 hr.). Models for polysaccharides, such as the disaccharides (307), were also cleaved under



(307)

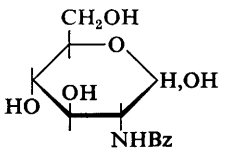
these conditions.[347] These results were compared with the hydrolysis of the corresponding unblocked amino-compounds which was slow and largely incomplete after 24 hr. The corresponding *N*-acetyl derivatives were hydrolysed more rapidly than (307), but the reaction did not go to completion because of concomitant cleavage of the acetamido-group to amino by the acid; the β-anomer of (307) was cleaved faster. It would appear, therefore, that the dinitrophenyl group has potential use in structural studies on aminopolysaccharides.

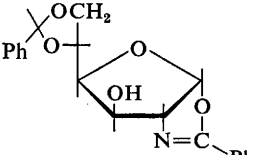
Experiments on the partial esterification of some 3-acetamido-3-deoxy-glycosides are described elsewhere in this Report.[209,210]

The preparation of 1,2-oxazoline derivatives of pyranoid compounds by way of 2-acylamino-glycosyl chlorides has been applied in the D-glucose,

[347] P. F. Lloyd and B. Evans, *J. Chem. Soc.* (*C*), 1968, 2753.

D-mannose, and chitobiose series.[348] During studies on the synthesis of 1,2-oxazolines from sugars containing free hydroxy-groups, the condensations (308)→ (309) and (310) → (311) were effected.[82] Methanolysis of (311)


(308)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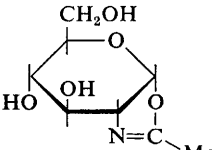
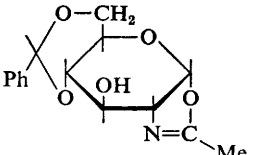
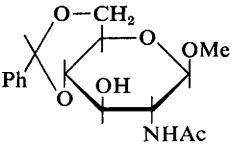
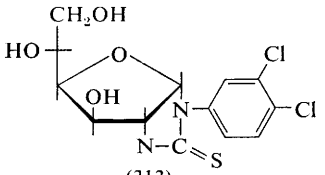

(310)

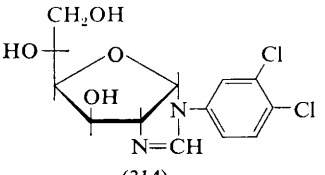

(311)


(312)

gave (312), and the corresponding *β-manno*-oxazoline afforded the *α-manno*-glycoside, but at an appreciably slower rate. The *gluco*-phenyloxazoline was more reactive than the methyl compound (311).

Treatment of the *N*-benzyl derivatives of 2-amino-2-deoxy-D-glucose, -D-galactose, and -D-mannose with aryl isothiocyanates in alkaline solution gave, in *e.g.* the first case, the imidazoline (313) and imidazolidene (314)


(313)


(314)

derivatives, which were shown by n.m.r. and oxidation studies to have furanoid structures.[349] A series of heterocyclic derivatives have been prepared by reaction of 4-chloro-5-nitropyrimidines with 2-amino-2-deoxy-D-glucose.[350]

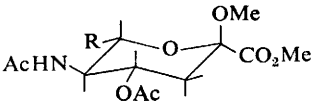
The radiolysis of 2-amino-2-deoxy-D-glucose and some of its derivatives has been investigated; no products were isolated but it was shown that different processes occurred at different sugar concentrations.[351]

[348] A. Ya. Khorlin, M. L. Shul'man, S. E. Zurabyan, and I. M. Privalova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2094.
[349] H. Fritz, C. J. Morel, and O. Wacker, *Helv. Chim. Acta*, 1968, **51**, 569.
[350] W. Pfleiderer, E. Bühler, and D. Schmidt, *Chem. Ber.*, 1968, **101**, 3794.
[351] L. A. Kudriashov, T. M. Senchenkova, L. I. Nedoborova, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1968, **38**, 2380.

Physical Measurements

The pK_a values of a variety of amino-sugars have been determined. Using these values, a set of parameters have been evaluated which permit the calculation of the pK_a values of other amino-sugars. The agreement between calculated and observed values was satisfactory.[352]

The n.m.r. spectra of several derivatives of *N*-acetylneuraminic acid have been examined, *e.g.* (315), which showed that these compounds had the



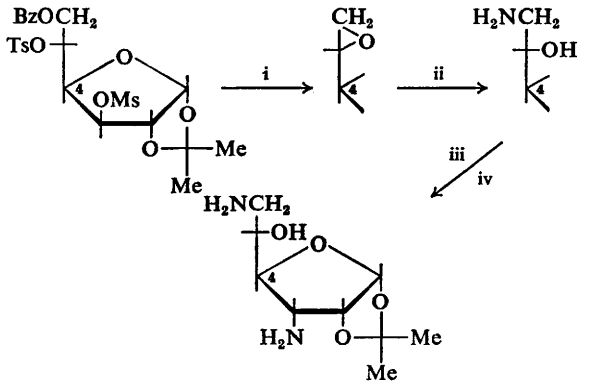
(315)

1*C* conformation. In some instances long-range coupling was used to assign the configuration at the anomeric centre (C-2).[353]

The o.r.d. spectra of methyl 2-acetamido-2-deoxyglycosides and of metal chelates of *N*-salicylidene derivatives of amino-sugars are discussed on p. 204.

Diamino- and Polyamino-sugars

The syntheses of methyl 4,6-diamino-4,6-dideoxy-α-D-glucoside and -galactoside have been reported.[276] Full details have appeared on the synthesis[354] of 2,3,4-triamino-1,6-anhydro-2,3,4-trideoxy-D-idose and on the synthesis[271] of derivatives of 2,3,4,6-tetra-amino-2,3,4,6-tetradeoxy-D-idose and -D-galactose; details of the sequences involved have been given previously (Vol. 1, p. 100).



(316)

Reagents: i, MeO^-; ii, NH_3; iii, NH_2NH_2; iv, H_2–Ni

Scheme 23

[352] S. Inouye, *Chem. and Pharm. Bull. (Japan)*, 1968, **6**, 1134.
[353] P. Lutz, W. Lochinger and G. Taigel, *Chem. Ber.*, 1968, **101**, 1089.
[354] F. W. Lichtenthaler and T. Nakagawa, *Chem. Ber.*, 1968, **101**, 1846.

3,6-Diamino-3,6-dideoxy-1,2-*O*-isopropylidene-β-L-talofuranose (316) has been obtained from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, *via* 6-*O*-benzyl-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-5-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose, as in Scheme 23.[355] Methanolysis of (316) gave the anomeric methyl pyranoside derivatives, whereas the same reaction on the per-acetyl derivative of (316) gave the β-pyranoside together with a product thought to be a talofuranoside derivative.

1″,6,6″-Triamino-1″,6,6″-trideoxyraffinose has been prepared by conventional methods *via* the triazido precursor.[356]

[355] J. Kovář and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 549.
[356] S. Umezawa and K. Tatsuta, *Bull. Chem. Soc. Japan*, 1968, **41**, 464.

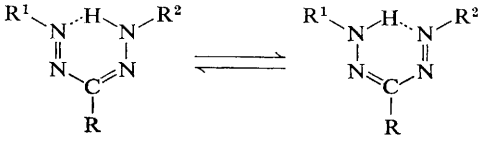
9

Hydrazones, Osazones, and Formazans

A survey of the literature of the reactions of sugars with hydrazine and its derivatives has appeared,[357] which summarises the literature up to the end of 1965.

The criticism (Vol. 1, p. 101) of the use of acetylation and of formazan formation for the investigation of the structure of phenylhydrazones has been answered by Mester.[358]

Two preliminary accounts have appeared describing the use of n.m.r. studies on ^{15}N-derivatives. In one it was shown that the chelate ring of formazans was the rapidly tautomerising system (317).[359] In the second, on the structure of osazones, the observations were not in accord with a classical hydrogen-bonded structure (318), but suggested that the chelate



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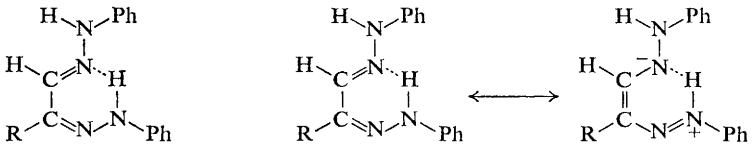


(318) (319)

ring was either a non-classical aromatic system, or a strongly conjugated structure with participation of the resonance forms (319).[360]

Many papers have again been published by El Khadem's group. They have described the synthesis of several mixed arylosazones derived from D-glucose, D-xylose, D-galactose, and L-sorbose, and their conversion into

[357] L. Mester, 'Dérivés hydraziniques des glucides' (Hydrazine derivatives of sugar s) 1967, Hermann, Paris, 179 pp.
[358] L. Mester and G. Vass, *Tetrahedron Letters*, 1968, 5191.
[359] L. Mester, A. Stephen, and J. Parello, *Tetrahedron Letters*, 1968, 4119.
[360] L. Mester, G. Vass, A. Stephen, and J. Parello, *Tetrahedron Letters*, 1968, 4053.

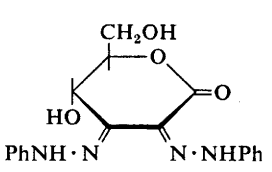
dianhydro-compounds of the Percival type and into pyrazole derivatives.[361] The products from the periodate oxidation of a large number of osazones have been isolated and characterised.[362] Phenylosazones of several heptoses have been converted into their 1-*N*-acetyl and penta-*O*-acetyl derivatives. Boiling them with acetic anhydride gave anhydro-compounds, similar to those prepared by Diels from hexose analogues, and also dianhydro-derivatives. Deacetylation of the penta-acetates with sodium hydroxide gave dianhydro-derivatives of the Percival type.[363]

It has been shown (*cf.* Vol. 1, p. 101) that 3,4,5,6-tetra-*O*-benzoyl-D-glucose phenylhydrazone on heating in oxygen-free aqueous dioxan gave the rearranged phenylazo-derivative, whereas heating in the same solvent in the presence of oxygen gave the phenylazo hydroperoxide.[363a]

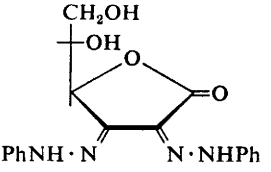
Several papers have also appeared on acylhydrazones from the same group, and the preparation of some 1-acylhydrazone 2-arylhydrazones and of some 2-acylhydrazone 1-arylhydrazones has been described.[364] A number of D-*arabino*-bis(aroylhydrazones) have been acetylated and oxidised to the corresponding 1-α-aroyloxyarylidene-1,2,3-triazoles.[365] When the reaction times were shortened, syntheses which yielded acyclic bis(benzoylhydrazones) (Vol. 1, p. 101) have been shown to give cyclic forms, characterised spectroscopically and chemically.[366] Further details have been published on the condensation of free sugars with substituted aroylhydrazones at different pH values.[367]

El Khadem's group have also studied the osazone of dehydro-L-ascorbic acid, and both preliminary details and full accounts of the work have been published. The structure (320) has been established for this compound by spectroscopic and chemical methods;[368, 369] this differs from the previously postulated structure (321) and showed that rearrangement to a δ-lactone ring occurred during the reaction. Treatment of (320) with mild oxidising agents, such as iodine, gave the anhydro-derivative (322).[370, 371] Reaction of (320) with alkali gave (323), whilst (322) gave (324).[371, 372]

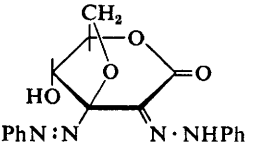
[361] H. El Khadem and M. M. A. Abdel Rahman, *Carbohydrate Res.*, 1968, **6**, 470.
[362] H. El Khadem, M. A. E. Shaban, and M. A. M. Nassr, *Carbohydrate Res.*, 1968, **8**, 113.
[363] H. El Khadem, M. M. A. Abdel Rahman, and M. A. E. Sallam, *J. Chem. Soc.* (*C*), 1968, 2411.
[363a] I. Dijong, *Z. Naturforsch.*, 1968, **23b**, 750.
[364] H. El Khadem, M. M. A. Abdel Rahman, and M. A. E. Shaban, *Carbohydrate Res.*, 1968, **6**, 465.
[365] H. El Khadem, M. A. M. Nassr, and M. A. E. Shaban, *J. Chem. Soc.* (*C*), 1968, 1465.
[366] H. El Khadem, M. H. Meshreki, G. H. Labib, and M. A. Nashed, *Carbohydrate Res.*, 1968, **8**, 243.
[367] H.-H. Stroh and H. Tengler, *Chem. Ber.*, 1968, **101**, 751.
[368] H. El Khadem and S. H. El Ashry, *Carbohydrate Res.*, 1968, **7**, 501.
[369] H. El Khadem and S. H. El Ashry, *J. Chem. Soc.* (*C*), 1968, 2247.
[370] H. El Khadem and S. H. El Ashry, *J. Chem. Soc.* (*C*), 1968, 2251.
[371] H. El Khadem and S. H. El Ashry, *Carbohydrate Res.*, 1968, **7**, 509.
[372] H. El Khadem and S. H. El Ashry, *J. Chem. Soc.* (*C*), 1968, 2248.



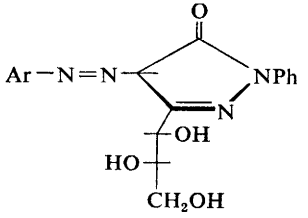
(320)



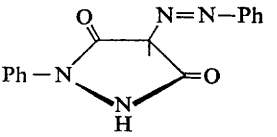
(321)



(322)



(323)



(324)

The syntheses of [β-(10-phenothiazinyl)-propionyl] hydrazones and their 2-chloro-analogues of aldoses have been reported.[373] The formation of hydrazones from the reaction of vicinal dimethanesulphonates with hydrazine is described on p. 133.

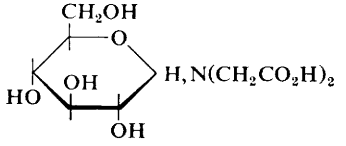
[373] H. Bräuniger and W. Delzer, *Die Pharmazie*, 1967, **22**, 680.

10
Miscellaneous Nitrogen-containing Compounds

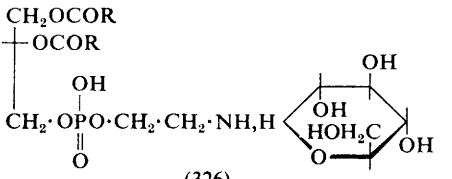
Glycosylamines

The periodate oxidation of glycosylamines has been reviewed.[374] Papers on glycosyl derivatives of ambident compounds such as 2-hydroxypyridine are discussed in Chapter 3 (p. 20).

The synthesis of *N*-*p*-carboxyphenyl- and *N*-*p*-chlorophenyl-glycosylamines of D-glucose, D-galactose, D-xylose, and L-arabinose has been described.[375] *N*-Glycosylpyrazoles of the same sugars and of D-mannose have been prepared by condensation between the appropriate acetobromosugar and pyrazole in nitromethane, followed by deacetylation.[376] Condensation of D-glucose with $NH(CH_2CO_2H)_2$ in the presence of sodium methoxide gave the glucosylamine (325).[377] Some phosphatidylethanolamine-*N*-glucosides, such as (326), have been synthesised as possible blood coagulants;[378] similar compounds have been isolated from natural sources.



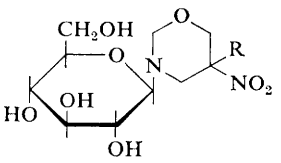
(325)



(326)

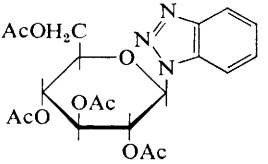
Condensation of D-glucosylamine with 2-alkyl-2-nitropropan-1,3-diols and formaldehyde gave 5-alkyl-3-(β-D-glucopyranosyl)-5-nitrotetrahydro-1,3-oxazines (327).[379] Reinvestigation of the products from the ammonolysis of penta-*O*-acyl derivatives of D-glucose has shown that as well as the previously isolated 1,1-bis(acylamido)-1-deoxyglucitols, cyclic *N*-acyl-β-D-glucosylamines were formed.[380] The results were compared with those previously obtained in the *manno*-series.

[374] Z. Fialkiewiczowa, *Zeszyty. Nauk Mat. Fiz., Chem.*, 1967, **7**, 179.
[375] B. N. Stepanenko, E. S. Volkova, and M. G. Chentsova, *Doklady Akad. Nauk S.S.S.R.*, 1967, **177**, 607; *Proc. Acad. Sci., U.S.S.R.*, 1967, **177**, 1084.
[376] J. Jasińska and J. Sokolowski, *Roczniki Chem.*, 1968, **42**, 275.
[377] P. Siegmund, *Z. physiol. Chem.*, 1967, **348**, 1505.
[378] J. D. Billimoria and K. O. Lewis, *Chem. Ind.*, 1968, 1731.
[379] I. Szczerek and T. Urbanski, *Carbohydrate Res.*, 1968, **7**, 357.
[380] A. S. Cerezo, J. F. Sproviero, V. Deulofeu, and S. Delpy, *Carbohydrate Res.*, 1968, **7**, 395.



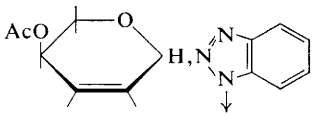
(327)

R = Me,Et or Pr

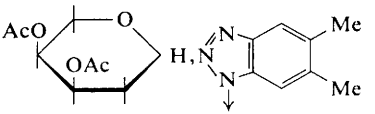


(328)

Glycosyl azides have been shown to react with benzyne (generated from anthranilic acid) to give benzotriazole derivatives. For example, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide gave (328) in 70% yield.[381] Similar derivatives of unsaturated sugars, such as (329), have been synthesised from the reaction of benzotriazole with acetylated glycals [3,4-di-*O*-acetyl-L-arabinal in the case of (329)] in the presence of trifluoracetic acid.[382] In the condensation of 5,6-dimethylbenzotriazole with the L-arabinal derivative a second product was also isolated, believed to be (330).

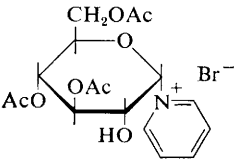


(329)

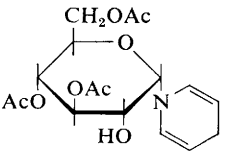


(330)

Borohydride reduction of the pyridinium compound (331) gave the dihydropyridine derivative (332);[383] analogues with methyl groups on the nitrogen-containing ring were also prepared.



(331)



(332)

The kinetics of the mutarotation of *N*-*p*-chlorophenyl-D-glucosylamine catalysed by variously substituted benzoic acids have been studied.[384] Kinetics of the same phenomenon catalysed by dipeptides were then

[381] G. García-Muñoz, J. Iglesias, M. Lora-Tamayo, and R. Madroñero, *J. Heterocyclic Chem.*, 1968, **5**, 699.
[382] M. Fuertes, G. García-Muñoz, M. Lora-Tamayo, R. Madroñero, and M. Stud, *Tetrahedron Letters*, 1968, 4089.
[383] A. Piskorska-Chlebowska, *Roczniki Chem.*, 1968, **42**, 309.
[384] T. Jasiński, K. Smiataczowa, and J. Sokolowski, *Roczniki Chem.*, 1968, **42**, 107.

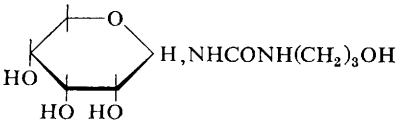
measured and the pK_a values of the catalysts were determined.[385] A study of the i.r. spectra of some *N*-arylglycosylamines has shown that they have the imine rather than the Schiff's base structure.[386]

As part of a study on the reaction of amino-compounds with free sugars, the decomposition of some *N*-alkylglycosylamines has been investigated. Compounds in the *xylo*- and *rhamno*-series gave the appropriate pyrrole-aldehyde, but such a compound was not formed in the *gluco*-series.[387] It was suggested that the *N*-substituted pyrrole-2-aldehyde derivatives were formed *via* a 3-deoxyglucosone intermediate.

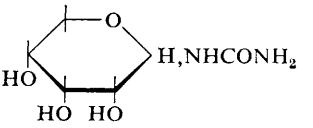
Treatment of 1-(β-D-ribopyranosyl)-indoles with boiling 80% acetic acid caused isomerisation, and after chromatography 1-(β-D-ribofuranosyl)-indole was isolated (7%).[388] The general method for determining the anomeric configuration of pyrimidine nucleosides (investigation of the n.m.r. spectrum after reduction of the 5,6-double bond) has been shown to be applicable to glycosyl indoles.[389] The synthesis of 1-(β-D-ribopyranosyl)- and 1-(β-D-ribofuranosyl)-indoles and -4-amino-indoles has been reported.[390]

Glycosyl-urea and -thiourea Derivatives

The ribose derivatives (333) and (334) were formed by borohydride reduction of the photohydration products of cytidine and uridine respectively.[391]



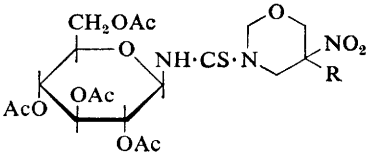
(333)



(334)

Hydrolysis of the derivatives (327) described above gave the free heterocyclic base, which was then condensed with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate to give the substituted thioureas (335).[379] The same glucose precursor has been used in the synthesis of the derivatives (336) and (337),[392] and (338).[393] The reaction of thiosemicarbazide with

[385] T. Jasiński, K. Smiataczowa, T. Sokolowska, and J. Sokolowski, *Roczniki Chem.*, 1968, **42**, 313.
[386] V. D. Shcherbukhin, B. N. Stepanenko, and E. S. Volkova, *Doklady Akad. Nauk S.S.S.R.*, 1967, **174**, 725.
[387] H. Kato, *Agric. and Biol. Chem. (Japan)*, 1968, **31**, 1089.
[388] M. N. Preobrazhenskaya, M. M. Bigdorchik, and N. N. Suvrov, *Khim. prirod. Soedinenii*, 1968, 128.
[389] R. J. Cushley, S. R. Lipsky, W. J. McMurray, and J. J. Fox, *Chem. Comm.*, 1968, 1611.
[390] E. Walton, F. W. Holly, and S. R. Jenkins, *J. Org. Chem.*, 1968, **33**, 192.
[391] N. Miller and P. Cerutti, *Proc. Nat. Acad. Sci., U.S.A.*, 1968, **59**, 34.
[392] C. Gmernicka-Haftek and W. Wieniawski, *Acta Pol. Pharm.*, 1967, **24**, 253 (*Chem. Abs.*, 1968, **68**, 69232y).
[393] W. Wieniawski and C. Gmernicka-Haftek, *Diss. Pharm. Pharmacol.*, 1968, **20**, 411.



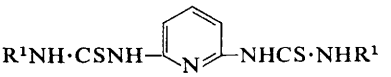
R = Me, Et, or Pr

(335)

$R^1NHCS \cdot NHR^2$

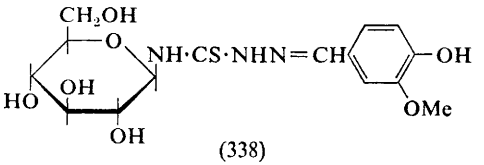
R^2 = Me, Et, or Bu

(336)



(337)

R^1 = β-D-Glucopyranosyl



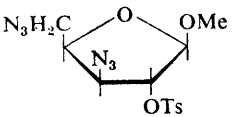
(338)

some simple dialdehydes has been studied as a model for its reaction with the dialdehyde from benzyl β-L-arabinopyranoside.[394]

Azides

Many papers have been described in Chapter 8 on the use of azido-sugars as intermediates in the synthesis of amino-sugars.

Gero and his group have continued their studies on polyazido-furanoid systems, and have synthesised the diazido-derivative (339) from methyl



(339)

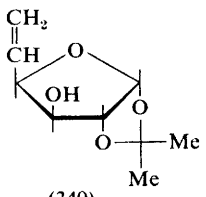
5-azido-5-deoxy-2,3-di-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside.[395] Other diazido-derivatives based on this skeleton have been prepared from the ring-opening reaction of an epimino-sugar (see below). Polyazido-derivatives in the 2,5-anhydropentitol series have been prepared [280] as shown in Scheme 16 (p. 64).

Addition of iodine azide to the unsaturated sugar (340) in acetonitrile gave the adduct (341) whose structure was established by *X*-ray crystallography;[396] addition occurred therefore in an anti-Markownikoff sense.

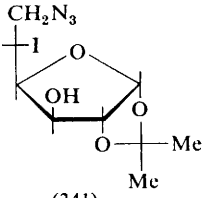
[394] V. C. Barry, J. E. McCormick, and R. S. McElhinney, *Carbohydrate Res.*, 1968, 7, 299.

[395] J. Cleophax, S. D. Gero, and J. Hildesheim, *Chem. Comm.*, 1968, 94.

[396] J. S. Brimacombe, J. G. H. Bryan, T. A. Hamor, and L. C. N. Tucker, *Chem. Comm.*, 1968, 1401.



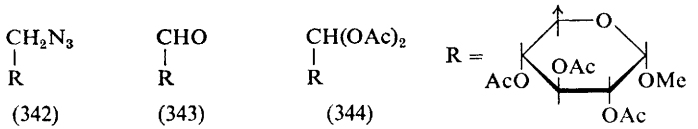
(340)



(341)

3,4,6-Tri-*O*-acetyl-D-glucal and -galactal gave, as yet uncharacterised, adducts.

Photolysis of methyl 6-azido-2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranoside (342) gave the 6-aldehydo-derivative (343),[397] isolated as its acetylated aldehydrol (344) or its 2,4-dinitrophenylhydrazone. The



(342) (343) (344)

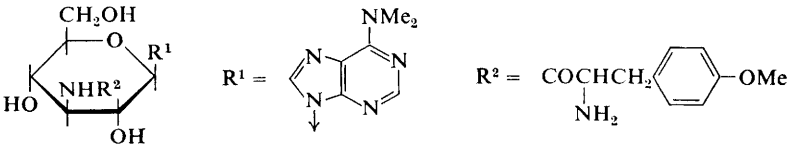
synthesis of 6,6′-diazido-6,6′-dideoxy-α,α-trehalose has been described.[279] Vinylic azides are discussed on p. 132.

The c.d. spectra of many azido-sugars have been recorded and an octant rule proposed.[398]

Nitro-compounds

The first naturally-occurring nitro-sugar (also branched chain) has been reported (see p. 138).

The dialdehyde–nitromethane cyclisation reaction has been extended to the synthesis of nucleotides bearing a 3′-amino-3′-deoxypyranosyl 5′-phosphate sugar moiety; both cytidine and uridine 5′-phosphate were successfully transformed in this way.[399] The puromycin analogue (345) has

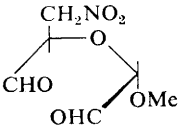


(345)

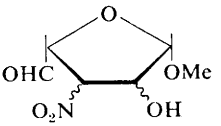
been synthesised by condensation of nitromethane with the dialdehyde derived from 6-dimethylamino-9-(β-D-ribofuranosyl)-purine, followed by standard steps.[400] A useful variant of the cyclisation reaction has been

[397] D. Horton, A. E. Luetzow, and J. C. Wease, *Carbohydrate Res.*, 1968, **8**, 366.
[398] H. Paulsen, *Chem. Ber.*, 1968, **101**, 1571.
[399] S. Takei and Y. Kuwada, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 944.
[400] F. W. Lichtenthaler and H. P. Albrecht, *Angew. Chem. Internat. Edn.*, 1968, **7**, 457.

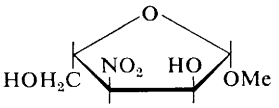
described by Baer's group,[401] and provides a novel route to 3-deoxy-3-nitro-glycofuranosides. Intramolecular cyclisation at pH 7·5 of the dialdehyde (346), obtained from periodate oxidation of methyl 6-deoxy-6-nitro-α-D-glucopyranoside, gave a mixture (347) of aldehydo-nitro-



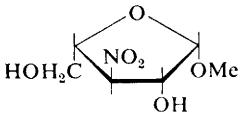
(346)



(347)



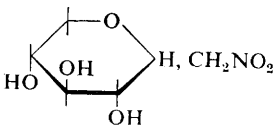
(348)



(349)

compounds having the β-L-configuration. After reduction of this mixture with sodium borohydride, compounds (348) and (349) were obtained and characterised by reduction and hydrolysis, and the resulting products were compared with known aminodeoxypentoses. This reaction should be of use in the synthesis of 3′-aminonucleoside derivatives. Other examples of nitromethane cyclisation reactions are given on pp. 101 and 167.

The reaction of D-xylose with nitromethane gave the 2,7-anhydroheptitol derivative (350) in high yield.[402] Two pairs of epimeric 2-acetamido-1,2-deoxy-1-nitroheptitols with the D-*glycero*-D-*talo*- and D-*glycero*-D-*galacto*-, and the D-*glycero*-L-*manno*- and D-*glycero*-L-*gluco*-configurations have been



(350)

synthesised by the nitromethane synthesis, and their configurations have been established by o.r.d. and c.d., as well as by chemical degradation.[333]

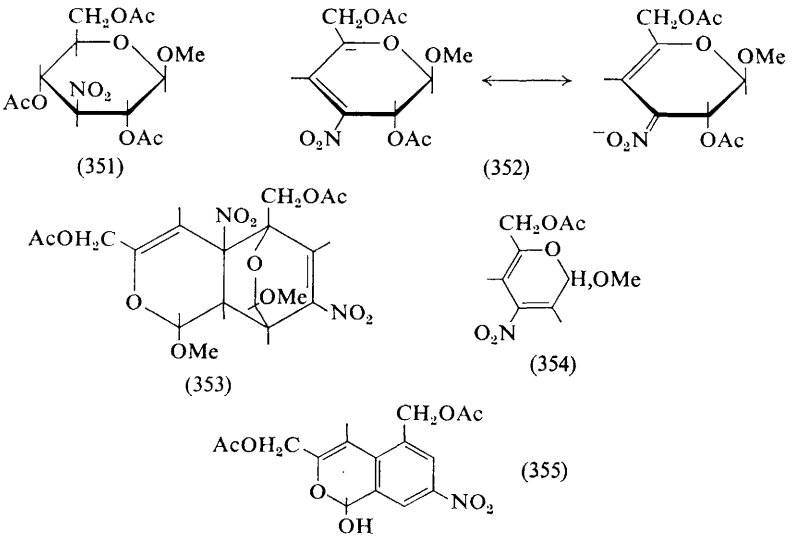
Two reports have appeared on the synthesis of nitro-compounds using unsaturated sugars as starting materials. Full details have now been given of the reaction of acetylated glycals with nitrosyl chloride to give 1,2-*cis*-2-deoxy-2-nitroso-α-D-aldopyranosyl chlorides in dimeric form, and the mechanism of the addition was discussed.[403] Analogous additions occur with dinitrogen tetroxide in ether at 0° to give, for example, dimeric

[401] H. H. Baer and I. Furić, *J. Org. Chem.*, 1968, **33**, 3731.
[402] R. N. Ray, *J. Indian Chem. Soc.*, 1968, **45**, 82.
[403] R. U. Lemieux, T. L. Nagabhushan, and I. K. O'Neill, *Canad. J. Chem.*, 1968, **46**, 413.

3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl nitrate from tri-*O*-acetyl-D-glucal. However, when methylene chloride was used as the solvent at −70°, 3,4,6-tri-*O*-acetyl-2-nitro-D-glycals were the main products. 3,4-Di-*O*-acetyl-D-xylal behaved differently and gave 2-nitro-D-xylal derivatives with nitrosyl chloride or dinitrogen tetroxide.

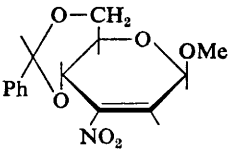
The addition of nitryl iodide to unsaturated sugars is described on p. 130.

Conditions for acetylating 3-deoxy-3-nitro-hexopyranosides have been investigated, as the usual reagents can cause epimerisation and elimination as well as simple esterification. Acetic anhydride in the presence of boron trifluoride has been shown to be a suitable system for such compounds.[404] Reaction of the acetylated derivative (351) with aqueous alkali in an attempted de-esterification caused elimination and the unsaturated nitronate (352) was produced, by loss of acetic acid. Dehydroacetylation of (351) with sodium hydrogen carbonate in benzene, and of its *manno*- and *galacto*-analogues gave the optically inactive product (353) by a Diels–Alder cyclisation of the intermediate (354). Compound (353) was characterised by n.m.r. and chemical methods which included thermal conversion into (355).[405]

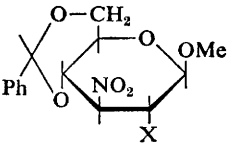


Treatment of nitro-olefins such as (356) (or their 2-*O*-acetyl-3-deoxy-3-nitro-glycoside precursors) with active hydrogen-containing reagents gave Michael adducts such as (357).[406] Preliminary results have been reported

[404] H. H. Baer, F. Kienzle, and F. Rajabalee, *Canad. J. Chem.*, 1968, **46**, 80.
[405] H. H. Baer and F. Kienzle, *J. Org. Chem.*, 1968, **33**, 1823.
[406] H. H. Baer and K. S. Ong, *Canad. J. Chem.*, 1968, **46**, 2511.



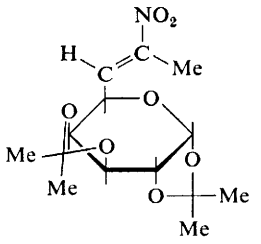
(356)



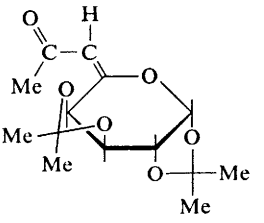
(357)

X = $CR^1R^2NO_2$
(R^1, R^2 = H or alkyl) or
$CH(CO_2Et)_2$

on the photolysis of the nitro-olefin (358), *cis*-6,7,8-trideoxy-1,2:3,4-di-*O*-isopropylidene-7-*C*-nitro-α-D-*galacto*-oct-6-enose. Irradiation gave a mixture of products of which three were characterised: one was the *trans*-isomer



(358)



(358a)

of (358) (20%); the other two were *cis*-6,8-dideoxy-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-oct-5-enos-7-ulose (358a) (8%) and its *trans*-isomer (6%), characterised spectroscopically.[407]

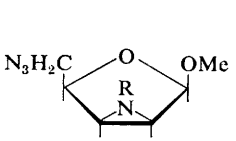
Epimino-sugars

Methyl 2,3,6-trideoxy-2,3-epimino-α-D-allopyranoside has been obtained by LAH reduction of methyl 2-benzamido-2-deoxy-3,4,6-tri-*O*-methanesulphonyl-α-D-glucopyranoside. The structure of the product was confirmed when it was also synthesised from methyl 2,3-acetylepimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-allopyranoside.[408]

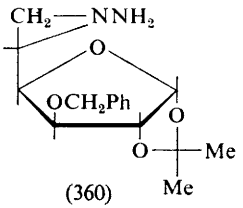
The methyl β-D-lyxofuranoside derivatives (359 and 359a) have been synthesised from the 3-azido-3-deoxy-2-toluene-*p*-sulphonyl-*xylo*-precursor. Ring-opening of (359) with sodium azide in DMF gave the *arabino*- and *xylo*-products in the ratio 1·8 : 1.[395] 5,6-Dideoxy-5,6-epimino-hexofuranose derivatives with the D-*gluco*-, L-*ido*-, and L-*altro*-configurations have been prepared from the appropriate *vic*-azido-toluene-*p*-sulphonyloxy-intermediates.[409]

[407] G. B. Howarth, D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Chem. Comm.*, 1968, 1349.
[408] C. F. Gibbs, L. Hough, A. C. Richardson, and J. Tjebbes, *Carbohydrate Res.*, 1968, **8**, 405.
[409] H. Saeki, T. Iwashige, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 188.

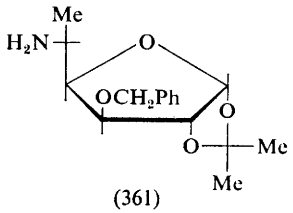
Treatment of 3-*O*-benzyl-1,2-*O*-isopropylidene-5,6-di-*O*-methanesulphonyl-α-D-glucofuranose with hot anhydrous hydrazine gave the 5,6-(*N*-amino-epimino) derivative (360) in high yield,[410] presumably formed by



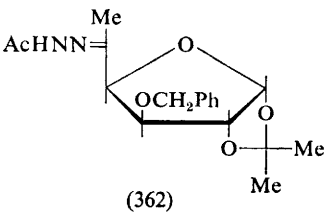
(359) R = Bz
(359a) R = H



(360)



(361)



(362)

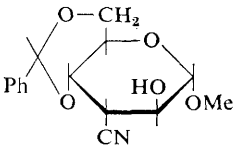
attack on the primary sulphonyloxy-group to give the 6-hydrazino-5-*O*-methanesulphonyl derivative which underwent ring closure. Hydrogenation of (360) in the presence of Raney nickel gave the 5-amino-5,6-dideoxy-derivative (361). The method was extended to provide syntheses of the L-*manno*- and L-*talo*-analogues of (361). Rearrangement of (360) in acetic anhydride and pyridine gave (362), opening up a new route to 6-deoxy-5-ulose derivatives. Attempts to extend the reaction of hydrazine with di-sulphonyl esters to di-secondary systems did not yield epimino-derivatives (see p. 133).

A further report on the puzzling ring-opening reactions of epimino-sugars has been published; in this case for the reactions of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-α-D-allopyranoside and its *N*-substituted derivatives.[411] Ammonium halides (except the fluoride) in DMF were used and with the free epimine gave the unexpected di-equatorial products, whereas the *N*-substituted compounds gave both di-equatorial and di-axial products, the latter predominating. With hydrogen halides, the unsubstituted epimine ring was stable enough to allow the preferential removal of the benzylidene ring, but with *N*-substituted derivatives ring-opening occurred before de-benzylidenation to give di-axial products. The reasons for this pattern of ring-opening (which must be contrasted with that reported for other nucleophiles) remain somewhat obscure.

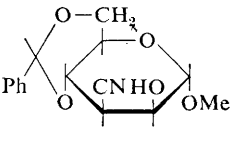
[410] H. Paulsen and D. Stoye, *Angew. Chem. Internat. Edn.*, 1968, **7**, 134.
[411] Y. Ali, A. C. Richardson, C. F. Gibbs, and L. Hough, *Carbohydrate Res.*, 1968, **7**, 255.

Other Nitrogen-containing Compounds

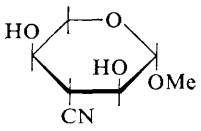
The reaction of aluminium triethyl and hydrogen cyanide in ether on epoxides has been shown to be a useful method for the synthesis of cyano-sugars (*cf.* Vol. 1, p. 39). Thus methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannoside gave the 3-cyano-3-deoxy-altroside derivative (363), which was epimerised by methanolic sodium methoxide to the *manno*-epimer (364).[412]



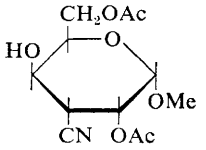
(363)



(364)



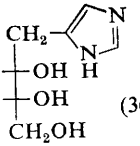
(365)



(366)

The structure of (363) was established by an *X*-ray analysis of its *p*-bromobenzenesulphonate ester. Methyl 2,3-anhydro-β-L-ribopyranoside and methyl 2,6-di-*O*-acetyl-3,4-anhydro-α-D-galactopyranoside gave the cyano-sugars (365) and (366), respectively.

Investigation of the reaction of aqueous ammonia with L-rhamnose has continued and several imidazole derivatives were isolated including 4-(*S*)-ethylimidazole, suggesting that the sugar is broken down *via* ethyl glyoxal.[412a] The major basic product from the reaction of ammonia and 3-*O*-substituted glucose derivatives has been shown to be L-*erythro*-4-(imidazol-4-yl)-butan-1,2,3-triol (367), which was believed to be formed by



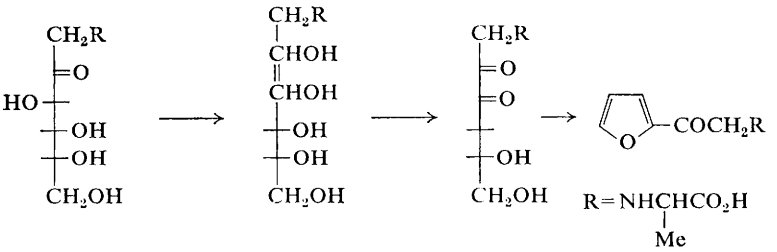
(367)

alkaline degradation of the hexose derivative to give 3-deoxy-D-*erythro*-hexulose which reacts in two ways: (*i*) by further alkaline cleavage to give formaldehyde and (*ii*) by reaction with ammonia and the formaldehyde to

[412] B. E. Davison, R. D. Guthrie, and A. T. McPhail, *Chem. Comm.*, 1968, 1273.
[412a] M. Komoto and H. Tsuchida, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 983.

give (367).[413] Deoxy-D-*erythro*-hexulose itself reacted to give (367), the mass spectrum of which was discussed in some detail.

Acid degradation of D-fructose-1-yl-amino acids, has been shown to give furan derivatives by the route illustrated in Scheme 24.[414]



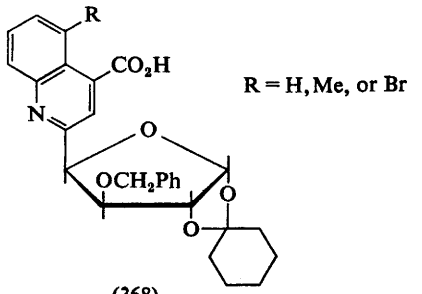
Scheme 24

The product from the condensation of thiocyanic acid with D-glucose has been shown to have a furanose structure,[415] and an independent study [416] has been made on the product from D-fructose (*cf.* Vol. 1, p. 111).

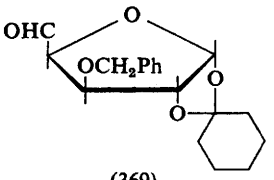
It has been reported that maltose phenylosotriazole was extremely easily hydrolysed at the glycosidic linkage, by the use of a water-soluble poly-(styrenesulphonic acid) resin at 95°, conditions under which maltose itself was not hydrolysed. It was suggested that this could form the basis of a step-wise degradation of polysaccharides; the reducing end unit could be converted into a phenylosotriazole, that unit removed by hydrolysis and so on.[416a] The n.m.r. and optical properties of aldose phenylosotriazoles have been studied (see pp. 196 and 205).

A variety of 1-aryl (or alkyl)-4-(D-*arabino*-tetrahydroxylbutyl)-imidazoles have been prepared from the corresponding imidazoline-2-thiones.[417] Several 3-*O*-benzyl-4-(4-carboxyquinol-2-yl)-1,2-*O*-cyclohexylidene-α-D-*xylo*-tetrofuranose derivatives (368) and one 4-(4-carboxybenzo[*f*]-quinol-2-yl) derivative have been prepared by application of the Doebner synthesis to 3-*O*- benzyl-1,2-cyclohexylidene-α-D-*xylo*-pentodialdose (369) using the appropriate arylamine followed by treatment with pyruvic acid.[418] Several 4-(polyhydroxyalkyl)-4-thiazoline-2-thiones have been described.[419]

[413] M. R. Grimmett, R. Hodges, and E. L. Richards, *Austral. J. Chem.*, 1968, **21**, 505.
[414] K. Heyns, J. Heukeshoven, and K.-H. Brose, *Angew. Chem. Internat. Edn.*, 1968, **7**, 628.
[415] J. Yoshimura and H. Hashimoto, *Bull. Chem. Soc. Japan*, 1968, **41**, 261.
[416] W. J. Humphlett, *Carbohydrate Res.*, 1968, **6**, 25.
[416a] J. N. BeMiller and D. R. Smith, *Carbohydrate Res.*, 1968, **6**, 118.
[417] J. Fernandez-Bolanos, M. Martin-Lomas, and D. Martinez-Ruiz, *Anales real Soc. Espan. fis. quim.*, 1968, **64**, 203.
[418] Yu. A. Zhdanov, Yu. E. Alexeev, and G. N. Dorofeenko, *Carbohydrate Res.*, 1968, **8**, 121.
[419] W. J. Humphlett, *Carbohydrate Res.*, 1968, **7**, 431.

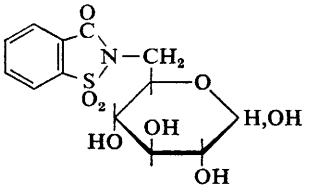


(368)



(369)

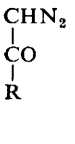
The *N*-(6-deoxy-D-glucos-6-yl)-saccharin compound (370) has been synthesised to see if it would lack the bitter after-taste associated with



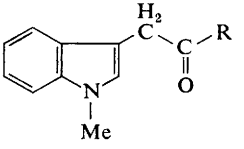
(370)

saccharin, or even perhaps to enhance its sweetness. In practice (370) was found to be strongly bitter with an unpleasant after-taste.[420]

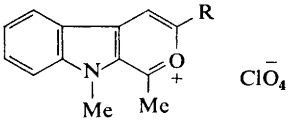
The oxocarbene formed from the copper-catalysed decomposition of the diazoketone (371) has been treated with *N*-methylindole to give 3,4,5,6,7-penta-*O*-acetyl-1-deoxy-(*N*-methylindol-3-yl)-*keto*-D-*gluco*-heptulose (372), which on treatment with acetic anhydride and perchloric acid underwent



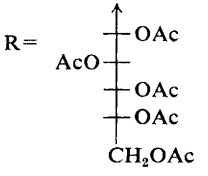
(371)

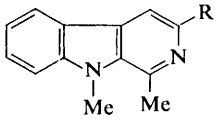


(372)



(373)



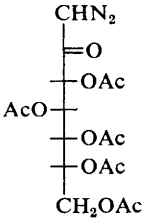


(374)

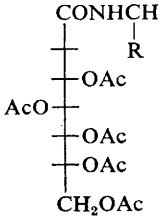
[420] E. M. Acton, J. E. Christensen, H. Stone, and L. Goodman, *Experientia*, 1968, **24**, 998.

cyclisation to the indole-[2,3-*c*]-pyrrylium perchlorate (373), further converted into the β-carboline (374) by ammonia.[421] Analogues of (373) and (374) were formed using other acid anhydrides.

The diazoketone (375), when treated with silver oxide and various amino-acid ethyl esters, gave the products (376).[422]



(375)

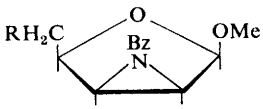


(376)

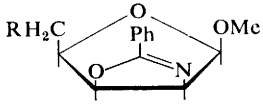
R = H, Me, or CH_2Ph

The use of oxazolines in the synthesis of glycosides has been described (see p. 18). The preparation of 1,2-oxazolines from 2-acylamino-2-deoxyglycosyl chlorides has been discussed.[348]

The oxazolines (197) and (198) have been obtained from the appropriate 3-benzamido-3-deoxy-2-*O*-toluene-*p*-sulphonyl derivatives.[286] Opening of the three-membered ring in the benzoylepimino-derivatives (377) gave the



(377)



(378)

R = OH or NHBz

oxazolines (378) as the major products, with (197) or (198) as the minor product as appropriate.[286]

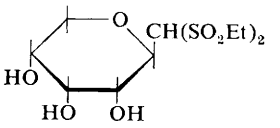
The o.r.d. spectra of heterocyclic compounds derived from aldoses are discussed on p. 204.

[421] Yu. A. Zhdanov, V. I. Kornilov, and G. N. Dorofeenko, *Carbohydrate Res.*, 1968, **6**, 414.

[422] Yu. A. Zhdanov, L. D. Shung, and V. I. Kornilov, *Zhur. obshchei Khim.*, 1968, **38**, 1411.

11
Thio-sugars

Oxidation of the diethyl dithioacetal of either D-allose or D-altrose gave bis(ethylsulphonyl)-β-D-ribopyranosylmethane (379), characterised by periodate oxidation studies and by n.m.r. studies on its tri-*O*-acetyl deriva-

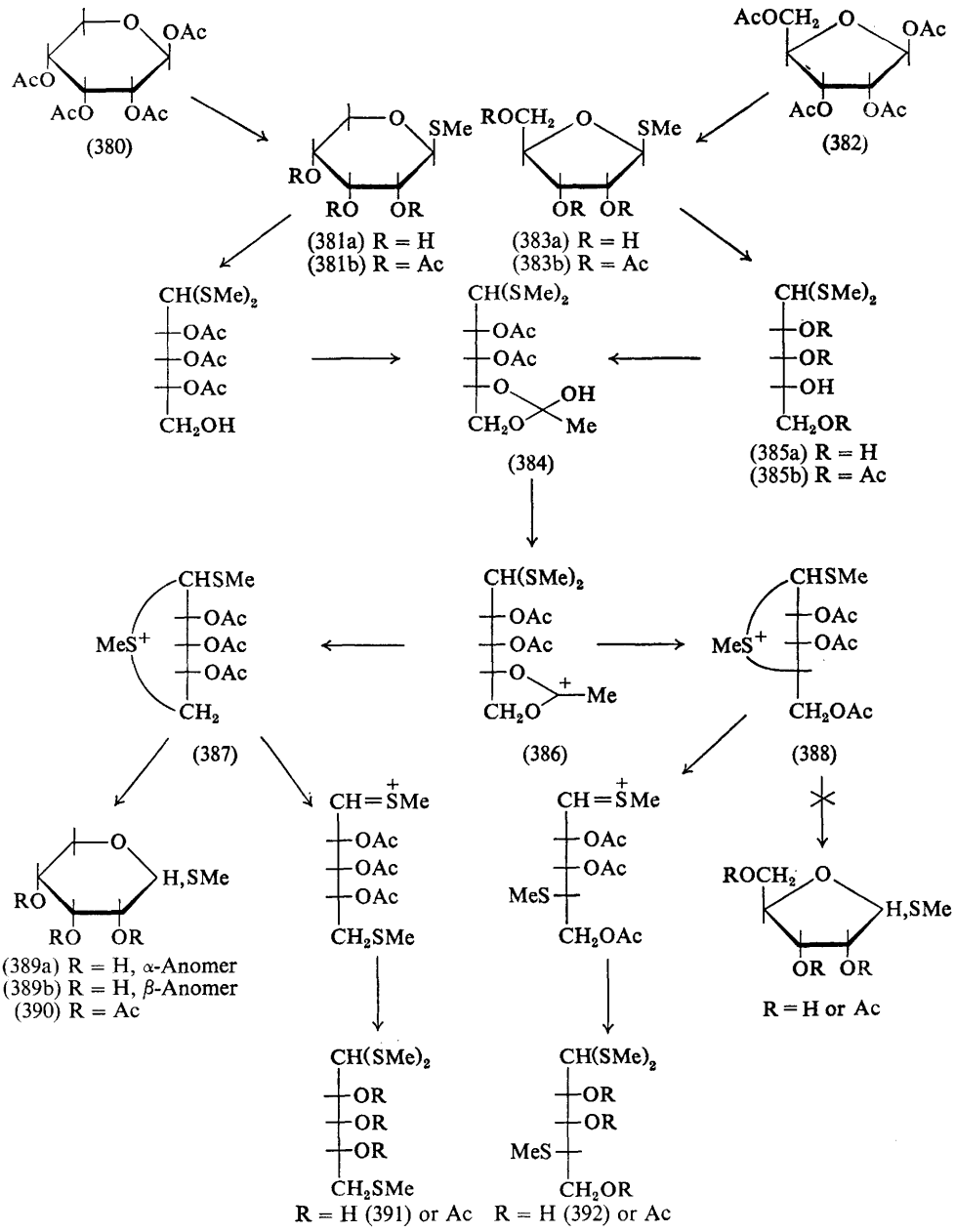


(379)

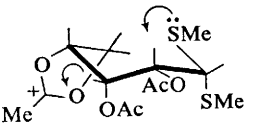
tive. The cleavage of (379) to D-ribose in ammonium hydroxide solution was compared with that of its 2-amino- and 2-acetamido-2-deoxy-derivatives. In the latter case, epimerisation to 2-acetamido-2-deoxy-D-arabinose occurred under the alkaline conditions used.[423]

Reactions of 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (380) with methanethiol in the presence of zinc chloride, and subsequent deacetylation gave the expected methyl 1-thio-β-D-ribopyranoside (381a), together with methyl 1,5-dithio-β-D-ribopyranoside (389b), and 4-*S*-methyl-4-thio-L-lyxose dimethyl dithioacetal (392), in addition to small amounts of 5-*S*-methyl-5-thio-D-ribose dimethyl dithioacetal (391). Similar treatment of 1,2,3,5-tetra-*O*-acetyl-ribofuranose (382) for short reaction times gave D-ribose dimethyl dithioacetal (385a) and small amounts of methyl l-thio-β-D-ribofuranoside (383a); longer reaction periods caused the complete disappearance of (383a), decrease of (385), and the appearance of two new main products (389b) and (392) together with a little (391).[424] These sequences are outlined in Scheme 25, which has the common intermediates (384) and (386); the difference in the reactions of the cyclic ions (387) and (388) is not at present understood. No compound (390) was found in the products. The formation of the β-anomer (389b) was believed to be sterically controlled, the intermediate for the formation of the α-anomer (389a) would be (393) with a destabilising 1,3-interaction.

[423] B. Coxon and L. Hough, *Carbohydrate Res.*, 1968, **8**, 379.
[424] N. A. Hughes, R. Robson, and S. A. Saeed, *Chem. Comm.*, 1968, 1381.

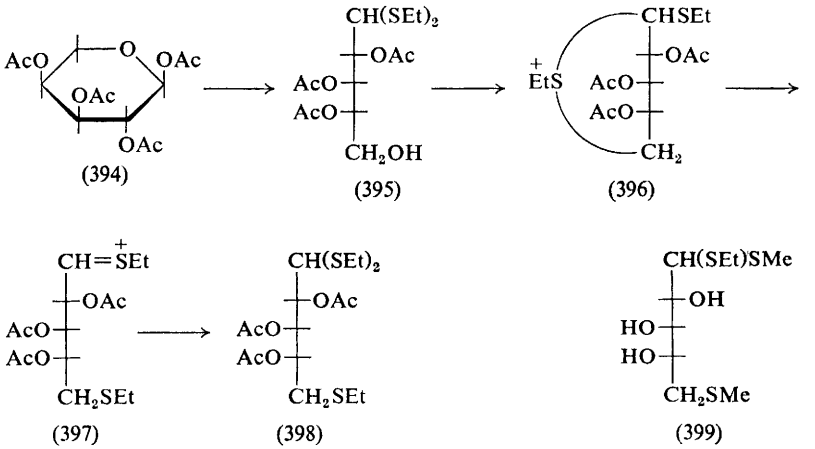

(380)
(381a) R = H
(381b) R = Ac
(382)
(383a) R = H
(383b) R = Ac
(384)
(385a) R = H
(385b) R = Ac
(386)
(387)
(388)
(389a) R = H, α-Anomer
(389b) R = H, β-Anomer
(390) R = Ac
R = H (391) or Ac
R = H (392) or Ac
R = H or Ac

Scheme 25



(393)

In view of these findings, the reaction of 1,2,3,4-tetra-*O*-acetyl-α-L-arabinopyranose (394) with ethanethiol and zinc chloride was reinvestigated.[425] The product was 2,3,4-tri-*O*-acetyl-5-*S*-ethyl-5-thio-L-arabinose diethyl dithioacetal (398), which arose *via* the ions (396) and (397) (Scheme



Scheme 26

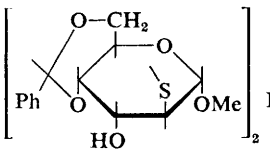
26) and not by direct displacement of the C-5-hydroxy-group in (395), as previously postulated. This was established by starting with the dithiomethyl analogue of (395) and allowing it to react with ethanethiol and zinc chloride to give (399), after deacetylation.

The reaction of D-xylose with ethanethiol in DMF has been studied in detail.[134]

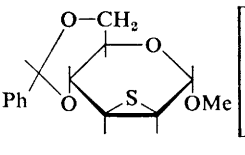
Reaction of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside with ammonium thiocyanate in 2-methoxyethanol gave bis(methyl 4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside)-2,2′-disulphide (400) (64%) and the *manno*-episulphide (401) (7%). It was shown that in a previously supposed synthesis of (401) (R. D. Guthrie and D. Murphy, *J. Chem. Soc.*, 1965, 6666), the product was in fact (400). Reaction of the anyhydro-alloside with the potassium salt gave only bis(methyl 4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside)-2,2′-sulphide (402). The best route for the

[425] N. A. Hughes and R. Robson, *Chem. Comm.*, 1968, 1383.

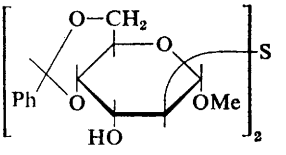
preparation of the episulphide (401) was by treating the 3-*O*-methanesulphonyl derivative of (400) with sodium borohydride in pyridine.[426] Reaction of the *allo*-epoxide with potassium ethyl xanthate (1 mol.) in methanol gave (402) (6%), the olefinic sugar (403) (9%), (404) (36%), and 5%



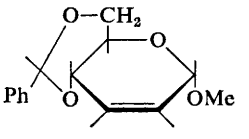
(400)



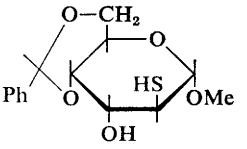
(401)



(402)



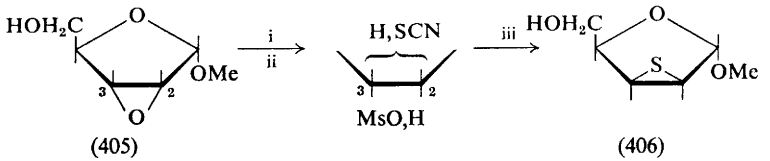
(403)



(404)

unchanged starting material, whereas 2 mol. of reagent gave (402) (57%) and (404) (36%); excess of reagent gave only (403). The analogous *manno*-epoxide with the same reagent in butan-1-ol gave (403) (50%) and in methanol, impure *allo*-episulphide. These results supported the hypothesis that the episulphide is an intermediate in the formation of olefins from epoxides.[426]

A preliminary report has described the syntheses of the *lyxo*-episulphide (406) from the epimeric *ribo*-epoxide (405) as shown in Scheme 27. Several



(405) (406)

Reagents: i, NH_4SCN–DMF; ii, MsCl–py; iii, ^-OMe

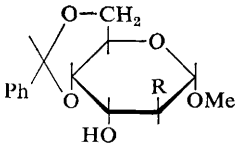
Scheme 27

5-*O*-derivatives were also described.[427] The *β-ribo*-episulphide was similarly prepared from the *β-lyxo*-epoxide. It was noted that the *α-lyxo*-epoxide was much less reactive towards ammonium thiocyanate than the *β*-anomer and that the *β*-anomer of (405) could not be opened even by using DMF at 110°. The opening of both (405) and the *β-lyxo*-epoxide occurred in *ca.* 2 : 1 ratio at C-3 and C-2. Investigations of the nature of the participation

[426] M. Kojima, M. Watanabe, and T. Taguchi, *Tetrahedron Letters*, 1968, 839.
[427] L. Goodman, *Chem. Comm.*, 1968, 219.

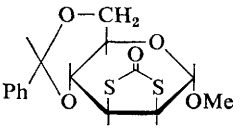
of *S*-benzoyl group in the displacement of a neighbouring sulphonyloxy-group have already been described [285] in this Report.

Ring-opening of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside with sodium *NN*-dimethyldithiocarbamate in acetone for 20 min. gave the *altro-NN*-dimethyldithiocarbamoyl derivative (407). Reaction in boiling methanol for 4 hr. gave mainly (407), but also a small amount of product believed to be the disulphide (400). The 3-*O*-toluene-*p*-sulphonyl

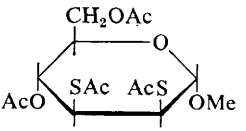


R = $SCSNMe_2$

(407)



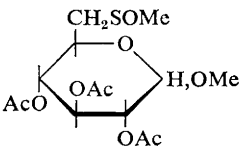
(408)



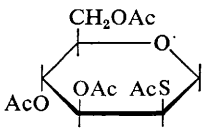
(409)

(or methanesulphonyl) derivative of (407) when treated with potassium acetate in ethanolic acetone gave the dithiocarbonate (408), reaction of which with sodium in liquid ammonia, followed by acetylation, gave the *cis*-2,3-dimercapto-derivative (409).[428]

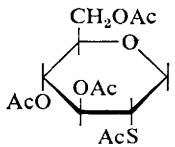
4,6-Dideoxy-4,6-dithiocyano-derivatives of several sugars have been described earlier in this Report,[270, 277] as have the corresponding 4,6-disulphides. Compounds (410) and (411) have been prepared from the corresponding toluene-*p*-sulphonates by reaction with thiomethylate ion, followed by acetylation and controlled oxidation. In the case of the α-anomer (410), diastereoisomeric sulphoxides were isolated by fractional crystallisation, but could not be assigned absolute configurations on the basis of their o.r.d. curves. Deacetylation of (410) occurred without racemisation at sulphur and one of the products was treated with acetic anhydride with the intention of effecting an intramolecular oxidation–reduction reaction to give a glycosidulose derivative (*cf.* DMSO–acetic anhydride oxidations). No ketonic products could be detected; instead α-acetoxylated sulphides were found. Attempts to simulate an oxidation of the Pfitzner–Moffatt type by use of DCC and pyridinium trifluoroacetate again gave no oxidation.[429]



(410) α-Anomer
(411) β-Anomer



(412)

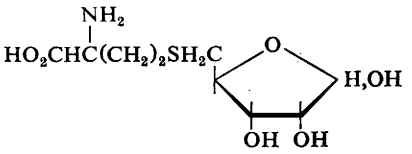


(413)

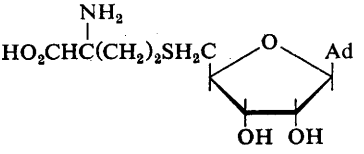
[428] S. Ishiguro and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1567.
[429] B. Lindberg and H. Lundström, *Acta. Chem. Scand.*, 1968, **22**, 1861.

Free radical addition[430] of thioacetic acid to 3,4,6-tri-*O*-acetyl-D-glucal gave two products (412) and (413) in the ratio 7:3.

S-(5-Deoxy-D-ribofuranos-5-yl)-homocysteine (414) has been obtained, specifically labelled in various centres, by enzymolysis of the adenosinyl analogue, which itself was prepared biochemically from adenosine and

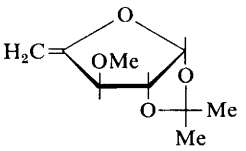


(414)

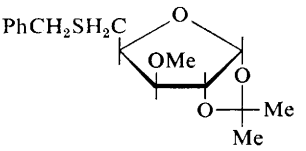


(415)

homocystein.[431] Treatment of cobalamine with the sodium salt of homocystein under u.v. light in the presence of sodium borohydride caused rupture of the C-5′—cobalt bond and gave (415).[432] Treatment of the unsaturated sugar (416) with benzyl mercaptan under u.v. light gave the adduct (417).[433] The synthesis of vinylic sulphur derivatives is described on p. 133.



(416)



(417)

[430] K. Igarashi and T. Honma, *Tetrahedron Letters*, 1968, 751.
[431] J. A. Duerre and C. H. Miller, *J. Labelled Compounds*, 1968, **4**, 171.
[432] A. M. Yurkevich, A. A. Amagaeva, and I. P. Rudakova, *Zhur. obshchei Khim.*, 1968, **38**, 1650.
[433] S. Inokawa, H. Yoshida, C.-C. Wang, and R. L. Whistler, *Bull. Chem. Soc. Japan*, 1968, **41**, 1472.

27
Alditols

D-Mannitol was found to be an important constituent of celery petioles and is believed to contribute largely to the flavour.[823] Several papers have appeared on crystallographic studies (see Chapter 24). Allitol has been oxidised to L-allulose,[824] and L-*glycero*-D-*galacto*-heptitol prepared by reduction of the aldose.[10] 1,3-Dideoxy-D-*ribo*-hexitol and its L-*lyxo*-isomer have been synthesised,[540] as has the novel cyclopropyl derivative (556).[511]

1,2:5,6-Di-*O*-bromoethylidene-D-mannitol has been described.[185] The following new derivatives of D-arabinitol have been prepared:[198] the 1,5-di-*O*-trityl compound and its triacetate and tribenzoate; the 1,5-di-*O*-toluene-*p*-sulphonyl ester and its tribenzoate and tri-*O*-methyl ether; and the 1,5-dideoxy-1,5-di-iodo tribenzoate. Several papers have appeared on phosphates and phosphites of D-mannitol.[242, 250, 251] 1,2,4,6-Tetra-*O*-benzoyl-D-glucitol has been described.[230]

2,3:4,5-Dianhydro-D-iditol[180] and 2,5:3,6-di-anhydro-D-glucitol[825] have been synthesised. Several 2-amino-2-deoxyalditols have been prepared.[333, 334] The mass spectra of several isopropylidene-alditols have been investigated.[712]

[823] R. Becker, *J. Food Sci.*, 1968, **33**, 128.
[824] J. G. Carr, R. A. Coggins, L. Hough, B. E. Stacey, and G. C. Whiting, *Phytochemistry*, 1968, **7**, 1.
[825] L. Vargha and J. Kuszmann, *Carbohydrate Res.*, 1968, **8**, 157.

12

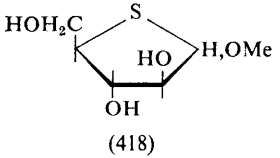
Derivatives with Sulphur or Nitrogen in the Sugar Ring

Compounds of this class have been reviewed.[434]

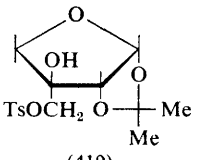
Sulphur Derivatives

The synthesis of the anomeric methyl 4-thio-D-arabinofuranosides (418) from the known 5-*S*-acetyl-3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-5-thio-α-D-glucofuranose has been described.[435]

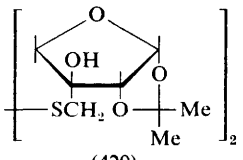
Treatment of the apiose derivative (419) with potassium thiolacetate in ethanol gave an almost quantitative yield of the 5,5′-disulphide (420), reduction of which with LAH gave the parent thiol; on methanolysis this gave the sulphur heterocycle (421), methyl 2,3-*O*-isopropylidene-4-thio-β-D-apio-D-furanoside, the first branched-chain derivative of this type.[195]



(418)



(419)



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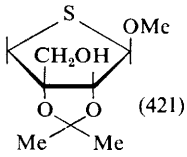
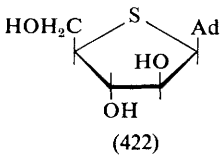
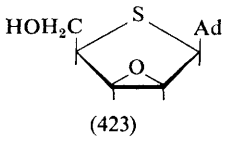


(421)



(422)



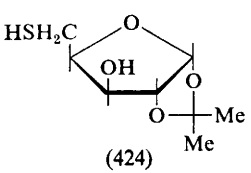
(423)

The Stanford group have continued their work on nucleosides bearing a hetero-atom sugar ring,[436] and the synthesis of the adenine derivative (422) has been described. In the ring-opening reaction of the epoxide precursor (423), opening at C-2 and C-3 occurred in the ratio 1 : 8, showing that

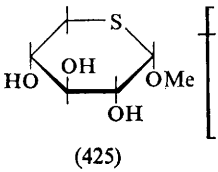
[434] H. Paulsen and K. Todt, *Adv. Carbohydrate Chem.*, 1968, **23**, 115.
[435] R. L. Whistler, U. G. Nayak, and A. W. Perkins jun., *Chem. Comm.*, 1968, 1339.
[436] E. J. Reist, L. V. Fisher, and L. Goodman, *J. Org. Chem.*, 1968, **33**, 189.

substituting the ring oxygen by sulphur does not change the mode of epoxide-opening.

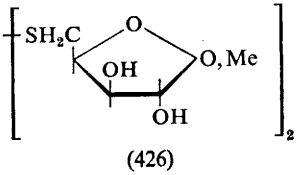
Methanolysis of 1,2-*O*-isopropylidene-5-deoxy-5-thio-α-D-xylofuranose (424) gave mainly methyl α-D-xylothiopyranoside (425), together with small amounts of the β-anomer of (425), bis(methyl 5-deoxy-D-xylofuranoside)-5,5′-disulphide (426), methyl 5-deoxy-5-thio-D-xylofuranoside (427), methyl 3,5-*O*,*S*-isopropylidene-5-deoxy-5-thio-D-xylofuranoside (428) as well as one other crystalline product. The structure of (428) was established by its synthesis from (427) by reaction with acidic methanol; 1,2:3,5-di-isopropylidene-5-deoxy-5-thio-D-xylofuranose was also formed in this



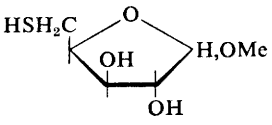
(424)



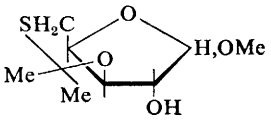
(425)



(426)



(427)



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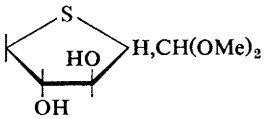
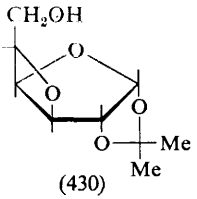


(429)

reaction.[437] The other product from the initial methanolysis was believed initially to be a mono-*O*-methyl derivative of methyl 5-deoxy-5-thio-xyloside and a number of compounds of this type were synthesised; none of them was identical with the unknown compound. Spectroscopic evidence suggested that it probably had the structure (429)[437] (*cf.* Vol. 1, p. 117 for an analogous selenium derivative).

Treatment of the oxetane (430) with benzylthiol anion in DMF at 150° gave the glucose derivative, which on reductive debenzylation gave the thiol (431), acid hydrolysis of which gave α-D-glucothiopyranose.[438] Thiosulphate anion also opened the anhydro-ring and provided an alternative route. Similar reactions were carried out in the D-xylose series. Thiopyranose derivatives formed from the reaction of ribose tetra-acetates with methanethiol and zinc chloride have already been described.[424]

[437] B. Nestadt and T. van Es, *Tetrahedron*, 1968, 1973.
[438] R. L. Whistler, T. J. Luttenegger, and R. M. Rowell, *J. Org. Chem.*, 1968, **33**, 396.



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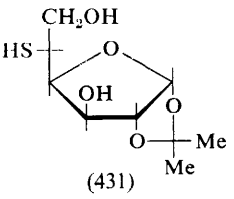
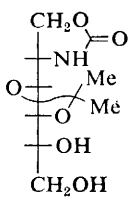


(431)

Nitrogen Derivatives

Compound (432), prepared from 2-amino-2-deoxy-3,4:5,6-di-*O*-isopropylidene-D-glucitol, has been cleaved with periodate, followed by removal of an isopropylidene group and ring closure to give (433), which formed an isopropylidene acetal. Removal of the carbamate group and the isopropylidene group gave the pyrrolidene-sugar (434), which was present in



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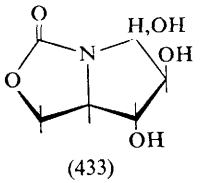

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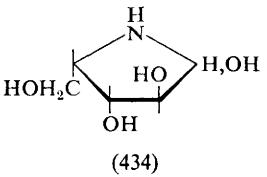
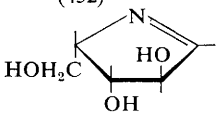


(434)



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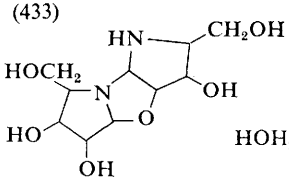
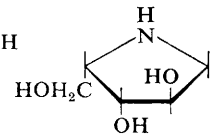


(436)

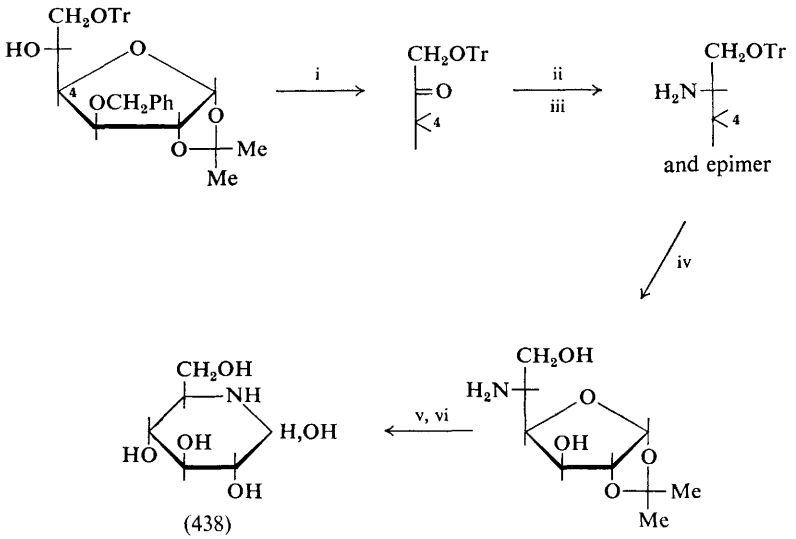


(437)

aqueous solution in equilibrium with the unsaturated sugar (435) and the dimer (436). *N*-Acetylation of the equilibrium mixture gave 4-acetamido-4-deoxy-L-xylopyranose, whilst reduction yielded the pyrrolidene (437). Azidolysis of 5-deoxy-2,3-*O*-isopropylidene-4-*O*-toluene-*p*-sulphonyl-D-arabinose diethyl acetal, reduction, and removal of the blocking groups gave analogous products.[439]

Two different syntheses by two different groups of Japanese workers of the antibiotic nojirimycin have been reported. In one report [440] the details of the original assignment of the D-glucopiperidinose structure (438) were given. Confirmation was obtained by its synthesis (in 22% overall yield from D-glucose in nine steps) the last part of which is shown in Scheme 28.

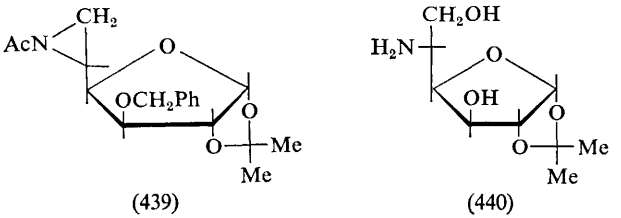
[439] H. Paulsen, J. Bruening, K. Propp, and K. Heyns, *Tetrahedron Letters*, 1968, 999.
[440] S. Inouye, T. Tsuruoka, T. Ito, and T. Niida, *Tetrahedron*, 1968, **24**, 2125.



Reagents: i, DMSO–Ac_2O; ii, NH_2OH–HCl–$KHCO_3$–MeOH; iii, Ni–NH_3–MeOH; iv, Li–NH_3; v, aq. H_2SO_3; vi, Dowex IX2

Scheme 28

The second synthesis used the opening of the 5,6-L-*gluco*-epimino-derivative (439) as the key step; acetic acid at 60° gave 5-acetamido-6-*O*-acetyl-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose, which was converted to (440) by standard methods. The latter compound was very

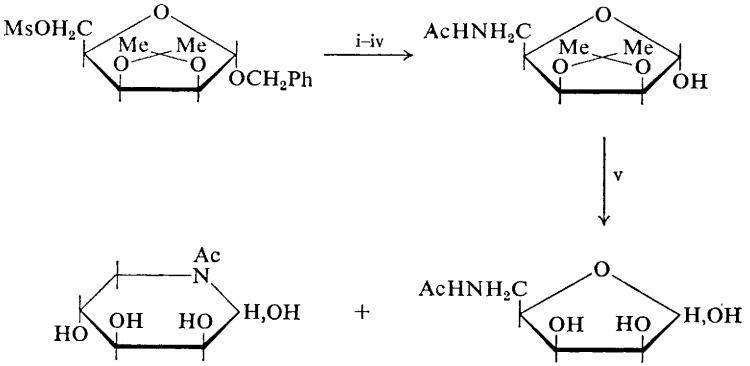


unstable to acids, but successful hydrolysis was achieved by converting it into its trifluoroacetyl derivative, followed by acid hydrolysis and then hydrolysis of the trifluoroacetyl groups on a basic resin to give amorphous (438), identical with the natural product.[441] Similar experiments were carried out on the *ido*-analogue of (439).

Benzyl 2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-α-D-lyxofuranoside has been converted into 5-acetamido-5-deoxy-D-lyxose, as shown in

[441] H. Sakei and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 962.

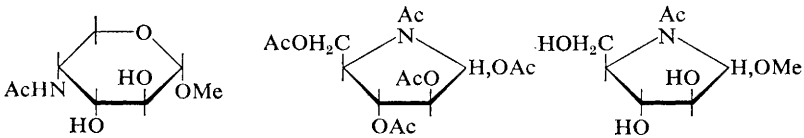
Scheme 29;[442] an alternative synthesis of this compound had been described previously (Vol. 1, p. 118). The pyranose isomer was isolated in crystalline form.



Reagents: i, NaN_3–DMF; ii, LAH; iii, Ac_2O–MeOH; iv, H_2–Pd/C; v, H_3O^+

Scheme 29

4-Acetamido-4-deoxy-D- and -L-arabinose and several five- and six-membered ring derivatives have been prepared, using the appropriate methyl 4-azido-4-deoxy-α-arabinopyranoside as starting material. The free sugars, in contrast to other 4-acetamido-4-deoxy-pentoses, were found to exist in aqueous solution in both five- and six-membered ring form. Acetolysis of (441) gave mainly the anomeric 4-acetamido-4-deoxy-D-arabinofuranose tetra-acetates (442) and from them the methyl furanosides



(443) were obtained by mild methanolysis, and deacetylation. Similar reactions were carried out in the L-series.[443]

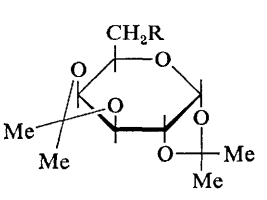
Methanolysis of the 5-amino-5-deoxy derivative (305) caused breakdown of the molecule to the pyridine derivative (306), presumably *via* a piperidinose intermediate.[345] In *N*-substituted 2-methylpiperidine derivatives, a steric interaction occurs when the methyl group is equatorial, so that in a series of such compounds it has been shown that the conformation with the methyl group axial is preferred.[444] This finding has important consequences in piperidinose chemistry.

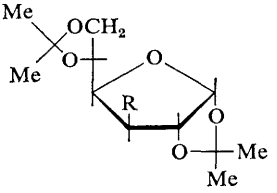
[442] J. S. Brimacombe, F. Hunedy, and M. Stacey, *J. Chem. Soc.* (*C*), 1968, 1811.
[443] A. J. Dick and J. K. N. Jones, *Canad. J. Chem.*, 1968, **46**, 425.
[444] H. Paulsen, K. Todt, and H. Ripperger, *Chem. Ber.*, 1968, **101**, 3365.

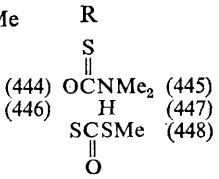
13
Deoxy-sugars

Reviews on various aspects of deoxy-sugar chemistry which formed the basis of an American Chemical Society symposium have been published in monograph form; the individual papers are referred to under their separate headings. Syntheses from aldulose and 2-hydroxyglycal derivatives,[445] and from *O*-acyldeoxyglycosyl halides[70] have been surveyed.

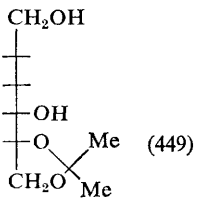
The photolysis of the dimethylthiocarbamates (444) and (445) gave the corresponding deoxy-sugars (446) and (447) together with the parent







(444) $OCNMe_2$ (445)
(446) H (447)
SCSMe (448)



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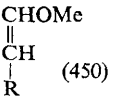


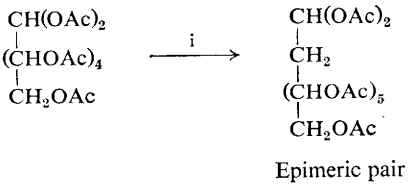
(450)

alcohols, and the latter could be re-esterified and the procedure repeated to give yields for the deoxy-compounds of 35–40%.[446] A new route of general interest for the synthesis of deoxy-sugars was therefore provided; other instances of its application were reported. Desulphurisation of thio-compounds as a means of obtaining deoxy-derivatives can be complex; a by-product obtained during the reduction of ester (448) has been shown to be the 2,3-dideoxyhexitol acetal (449).[447]

[445] W. G. Overend, *Adv. Chem. Ser. No.* 74, 1968, 141.
[446] R. H. Bell, D. Horton, and D. M. Williams, *Chem. Comm.*, 1968, 323.
[447] K. Kefurt, Z. Kefurtova, L. Dolejs, D. Snobl, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 931.

New routes to 2-deoxyaldoses have resulted from the application by Russian chemists of Wittig reagents, but the yields obtained do not recommend the method. Thus *aldehydo*-D-[448] and -L-[449] arabinose derivatives with methoxymethylenetriphenylphosphorane gave vinyl ether adducts (450) which on hydrolysis afforded 2-deoxyhexoses. In one study[448] D-glucose, D-mannose, and D-arabinose were obtained in addition to the desired 2-deoxy-D-*arabino*-hexose.

Another novel condensation utilised peracetylated-*aldehydo*-sugars (D-glucose and D-mannose) and vinyl acetate in the presence of boron trifluoride etherate (Scheme 30). Acid hydrolysis of the products gave

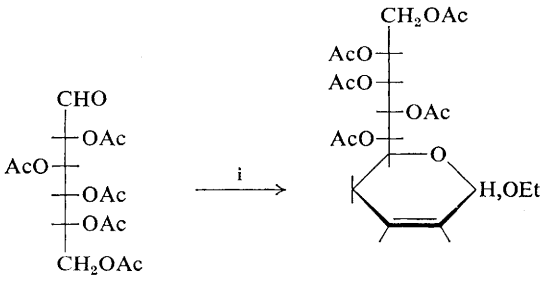


Epimeric pair

Reagent: i, CH_2=CHOAc–BF_3

Scheme 30

the free 2-deoxy-sugars but ammonia removed all acetyl groups except that at C-1. An interesting extension of this reaction is illustrated in Scheme 31.[449a]



Reagent: i, CH_2=CH·CH=CHOEt

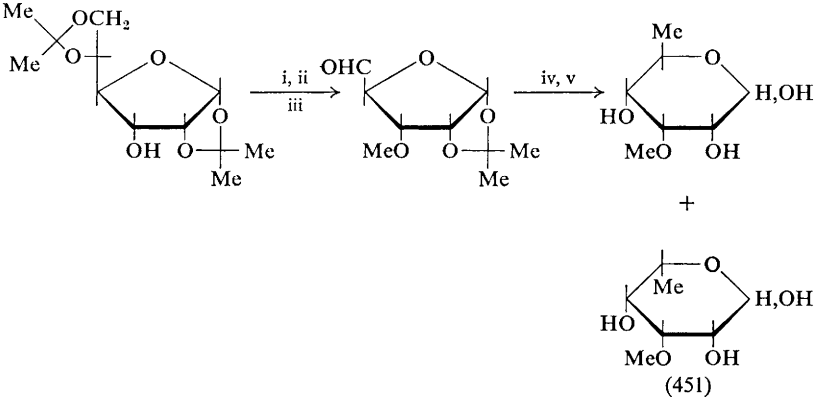
Scheme 31

Other deoxyaldoses which have been prepared are 2-deoxy-D-*ribo*-hexose[332] and 2-deoxy-D-*galacto*-heptose,[450] both by application of the standard nitromethane synthesis.

[448] B. A. Dmitriev, N. N. Aseeva, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 1342.
[449] Yu. A. Zhdanov and V. G. Alekseeva, *Zhur. obshchei Khim.*, 1968, **38**, 2594.
[449a] Yu. A. Zhdanov, L. A. Uzlova, and K. V. Gaponova, *Zhur. obshchei Khim.*, 1968, **38**, 2618.
[450] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1968, **46**, 789.

4-Deoxy-sugars, together with 2-deoxy-ald-3-uloses, were found amongst the radiolysis products of cellobiose and lactose in the absence of oxygen; mechanistic schemes for the degradations were proposed.[451] Other 4-deoxyaldohexose derivatives have been synthesised from racemic starting materials.[175]

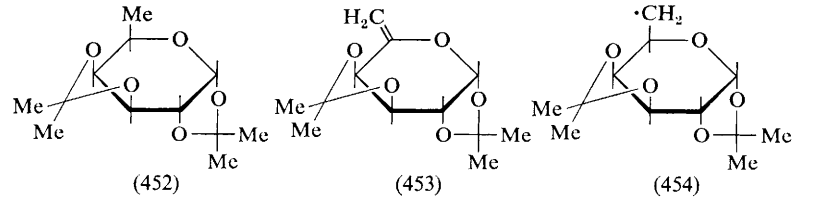
L-Acovenose, a rare naturally-occurring sugar found in plant glycosides, has been finally characterised as 6-deoxy-3-*O*-methyl-L-talose (451) by its synthesis as outlined in Scheme 32.[452] Photochemical reactions



Reagents: i, MeI–Ag_2O–DMF; ii, H_3O^+; iii, IO_4^- iv, MeMgBr; v, H_2O

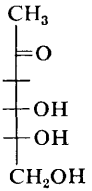
Scheme 32

can also provide a means of obtaining 6-deoxyhexose derivatives. Irradiation of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-D-galactose in methanolic sodium methoxide gave complete reaction in 2 hr. and the 6-deoxy-analogue (452) was obtained in 83% yield. Photolysis in a poor hydrogen-donating solvent gave (452) in only 32% yield together with (453) (36%) in reactions assumed to proceed *via* the radical (454).[453]



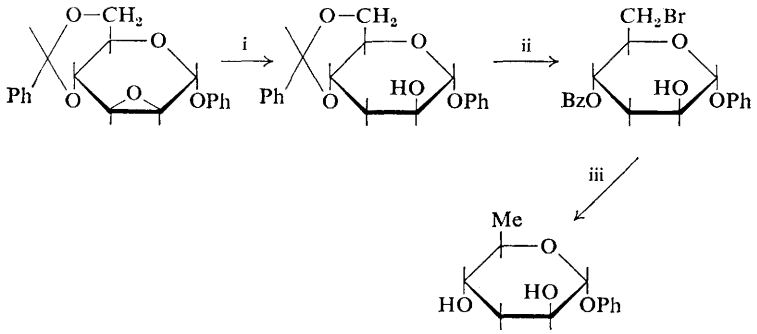
In the area of dideoxy-sugars, compound (455) has been synthesised as the first step in the preparation of nucleosides in which the anomeric

[451] N. K. Kochetkov, L. A. Kudriashov, S. M. Yarovaya, E. I. Bortsova, and O. S. Chizhov, *Zhur. obshchei Khim.*, 1968, **38**, 2234.
[452] B. M. Kapur and H. Allgeier, *Helv. Chim. Acta*, 1968, **51**, 89.
[453] W. W. Binkley and R. W. Binkley, *Carbohydrate Res.*, 1968, **8**, 370.



(455)

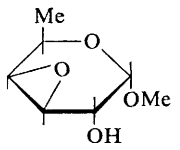
hydrogen is replaced by methyl. The method employed was based on the diazomethane reaction with either 3,4,5-tri-*O*-benzoyl-2-deoxy-D-*erythro*-pentose or the corresponding pentonyl chloride followed by reduction, and the ketose was characterised as its crystalline 6-*O*-(1-adamantoyl)-dimethyl dithioacetal.[454] Tyvelose has been prepared as its phenyl α-D-glycoside as shown in Scheme 33.[455]



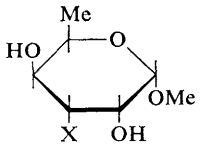
Reagents: i, LAH; ii, NBS–CCl_4; iii, LAH

Scheme 33

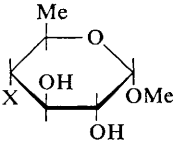
With a view to preparing 3,6-dideoxy-D-*xylo*- and 4,6-dideoxy-D-*xylo*-hexose a number of ring-opening reactions of (456) have been studied. Reductive ring-opening with LAH–THF gave (457) and (460) in the ratio 60 : 1; for Raney nickel this ratio was 1 : 2. With hydrobromic acid (458) and (461) were formed in the ratio 3 : 1 and sodium butylthiolate gave (459) and (462) in the ratio 1 : 2; from these products the deoxy-analogues



(456)



	X	
(457)	H	(460)
(458)	Br	(461)
(459)	SBu	(462)

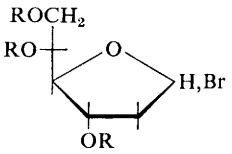


[454] B. F. West, K. V. Bhat, and W. W. Zorbach, *Carbohydrate Res.*, 1968, **8**, 253.
[455] S. Svensson, *Acta Chem. Scand.*, 1968, **22**, 2737.

(457) and (460) were obtained by reduction.[456] A specific synthesis of 4,6-dideoxy-D-*xylo*-hexose was provided by the reductive desulphurisation of several 4,6-dithio-derivatives.[277]

Two disaccharides isolated from plant glycosides and named pachybiose and asclepobiose have been characterised as 2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl)-3-*O*-methyl-D-*arabino*- and -D-*ribo*-hexose respectively. N.m.r. techniques were used extensively and a series of useful spectra were recorded.[457] Further, two related compounds drebyssobiose and lilacinobiose have been shown to be 2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl)-D-*ribo*-hexose and 2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-D-glucopyranosyl)-3-*O*-methyl-D-*ribo*-hexose, respectively.[458]

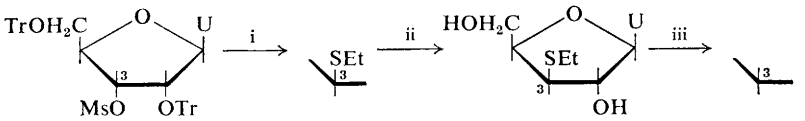
Various developments have been recorded in the field of deoxynucleosides. Treatment of methyl 2-deoxy-D-*ribo*-hexofuranoside with *p*-nitrobenzoyl chloride followed by hydrobromic acid gave the crystalline (463) which was converted into 1-(2-deoxy-D-*ribo*-hexofuranosyl)thymine.[459]



R = *p*-$NO_2 \cdot C_6H_4 \cdot CO$

(463)

Several workers have studied the synthesis of 3′-deoxynucleosides. The nucleophilic displacement of 3′-sulphonyloxy-groups for this purpose has been assessed as unsuitable generally although reports of successful applications have appeared (see below). A preferred route involves treatment of 2′,5′-disubstituted compounds with triphenylphosphite methiodide and reduction of the resulting deoxy-iodo-derivatives. It was noted that acyl migration can occur during iodinations, thus limiting the choice of the 2′,5′-protecting groups.[460] 3′-Deoxyuridine has been synthesised as shown in Scheme 34, but complications which limit the value of this approach (see above) included the formation of the anhydronucleoside

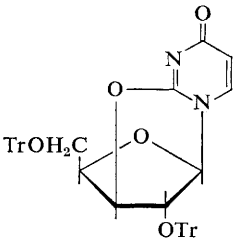


Reagents: i, NaSEt; ii, H_3O^+; iii, H_2–Ni

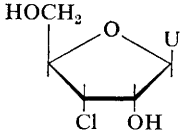
Scheme 34

[456] K. Čapek, J. Němec, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 1758.
[457] H. Allgeier, *Helv. Chim. Acta*, 1968, **51**, 311.
[458] H. Allgeier, *Helv. Chim. Acta*, 1968, **51**, 668.
[459] C. C. Bhat, K. V. Bhat, and W. W. Zorbach, *Carbohydrate Res.*, 1968, **8**, 368.
[460] G. A. R. Johnston, *Austral. J. Chem.*, 1968, **21**, 513.

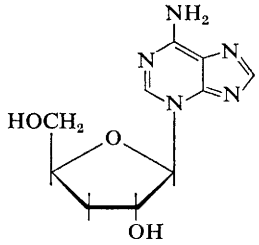
(464), which on treatment with hydrogen chloride gave the chlorinated nucleoside (465).[461]



(464)

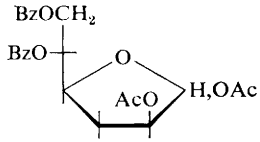


(465)

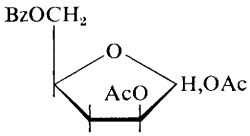


(466)

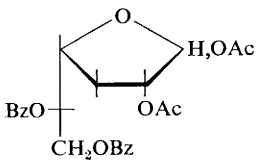
Syntheses of 3′-deoxynucleosides have been effected by use of 3-deoxyglycosyl halides and by this general approach a nucleoside believed to be the cordycepin isomer (466) was obtained.[462] Similarly, a series of 3-deoxyglycosyladenines was synthesised using the deoxyglycosyl derivatives (467)–(469).[463]



(467)



(468)



(469)

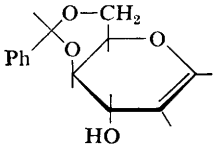
[461] G. Kowollik and P. Langen, *Chem. Ber.*, 1968, **101**, 235.
[462] N. J. Leonard and M. Rasmussen, *J. Org. Chem.*, 1968, 2488.
[463] J. Prokop and D. H. Murray, *J. Pharm. Sci.*, 1968, **57**, 1697.

14
Unsaturated Derivatives

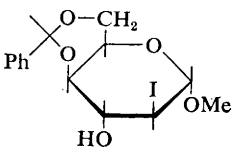
This area of research continues to be one of the most active growth points in monosaccharide chemistry, and 1968 saw appreciable developments. A paper referring to the first study of the mass spectrometry of unsaturated compounds is noted on p. 199, and the application of the 'oxo' reaction has been reviewed.[301a]

Glycals

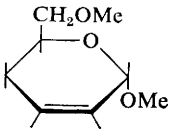
An anomaly in the literature on the products formed when methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside (77) is treated with methyl-lithium has been shown to arise as a result of variable purity of the reagent.[171] Other workers rationalised the situation along similar lines and have developed a synthesis of 4,6-*O*-benzylidene-D-gulal (470) from (471).[464] Tri-*O*-acetyl-D-glucal is the major product formed on treating Brigl's anhydride with sodium cobalt tetracarbonyl and carbon monoxide in methanol (see p. 157).



(470)



(471)

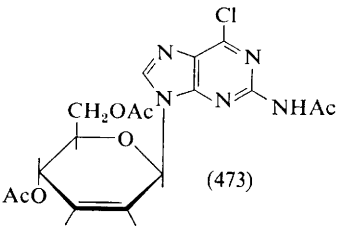


(472)

Details of the important reaction (previously described in outline) undergone by acetylated glycals with nitrosyl chloride to give acetylated 1,2-*cis*-2-deoxy-2-nitroso-α-D-aldopyranosyl chlorides in dimeric form have been published in full, and the mechanism of the addition was considered.[403] The results are reported more fully on p. 84 and applications of the products in glucoside syntheses are described on p. 10. Other additions to glycal esters are recorded in Chapter 7; uncharacterised adducts were obtained with iodine azide.[396] The methoxybromination of 3,4-dihydro-2-methoxymethyl-(2*H*)pyran has been utilised in the synthesis of (472) (racemic).[168]

[464] R. U. Lemieux, E. Fraga, and K. A. Watanabe, *Canad. J. Chem.*, 1968, **46**, 61.

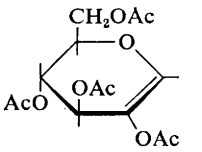
A general method involving the use of boron trifluoride as a catalyst for converting tri-*O*-acetyl-D-glucal into 2,3-unsaturated glycosides has been described,[63] and has the advantage of using equivalent proportions of glycal and alcohol, and of affording the α-anomers in *ca.* 90% stereochemical purity. The application of the products to the synthesis of saturated α-glycosides has already been referred to.[63] The same type of reaction (but with trifluoroacetic acid as catalyst) was applied to 3,4-di-*O*-acetyl-D-xylal and -L-arabinal in the presence of benzotriazole; addition products were, however, also observed.[382] Similarly, tri-*O*-acetyl-D-glucal fused with 2-acetamido-6-chloropurine in the presence of trifluoroacetic acid gave



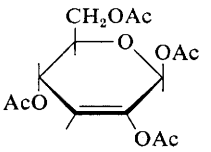
(473)

(473) in 30% yield,[465] but fusion of 6-chloropurine with 3,4-di-*O*-acetyl-D-arabinal in the presence of an acid catalyst gave mainly the addition products 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-1-α- and -β-D-*erythro*-pentopyranosyl)purine. The structures of these products were confirmed by independent syntheses, and the configurations were assigned by detailed n.m.r. studies. The o.r.d. curves of compounds in this series were described.[466]

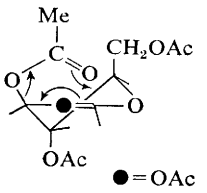
In the area of glycal derivatives carrying substituents on the vinylic carbon atoms it was observed that on heating in inert solvents (nitrobenzene used), esters of 1-deoxyald-1-enopyranoses, in which the C-3–C-4 relationship was *trans*, rearranged stereospecifically to give the isomeric 3-deoxyald-2-enopyranose isomers, which have the group at C-1 on the same side of the ring as was the C-3 group of the starting material; for example, (474) rearranged to (475), and an S_Ni'-mechanism (476) was considered to



(474)



(475)

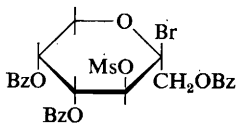


(476)

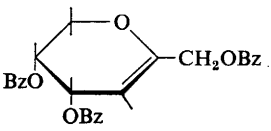
[465] E. E. Leutzinger, R. K. Robins, and L. B. Townsend, *Tetrahedron Letters*, 1968, 4475.
[466] E. E. Leutzinger, W. A. Bowles, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1968, **90**, 127.

operate. Compounds with the C-3–C-4 *cis*-relationship (galactal derivatives, for example), did not isomerise under these conditions. All compounds of the hydroxyglycal series, however, did undergo this isomerisation in the presence of boron trifluoride, but under these conditions, anomerisation of the products occurred and equilibrium mixtures of α,β-products were obtained.[467]

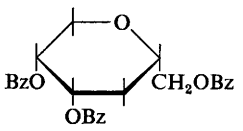
For the first time, the synthesis of a glycal with a carbon substituent at C-1, *i.e.* one derived from a ketose, has been described. Benzoylation of 3-*O*-methanesulphonyl-D-fructose gave two tetra-esters, one of which afforded the bromide (477) which on treatment with sodium iodide in acetone gave (478), characterised by hydrogenation to the known glycal (479).[468]



(477)



(478)



(479)

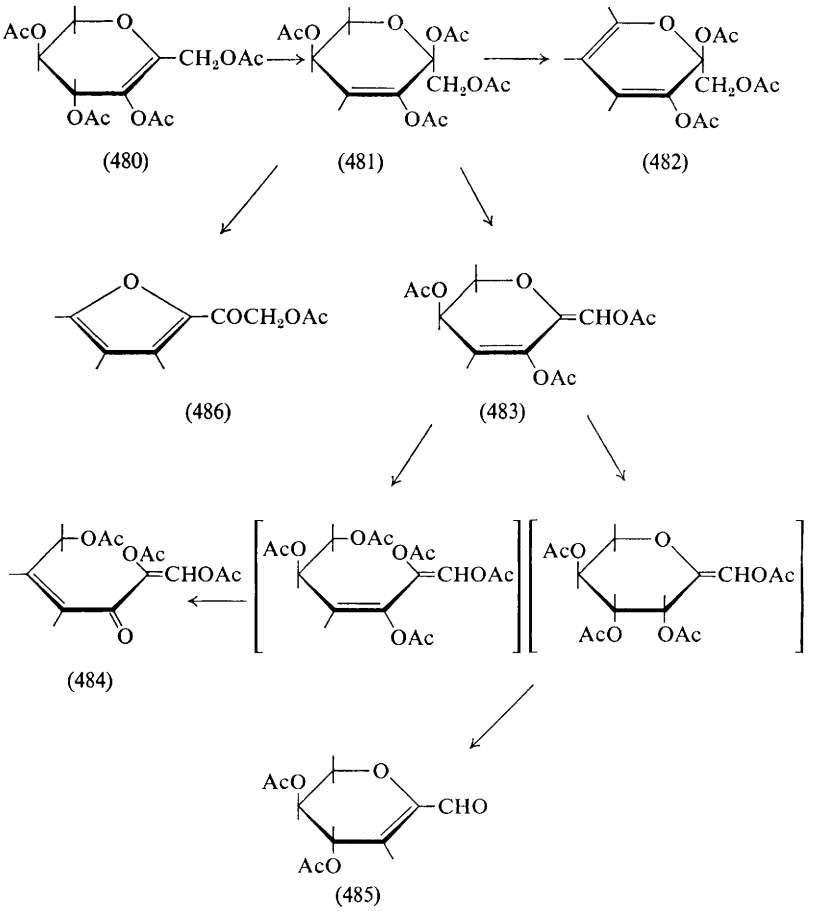
Other workers, in a lengthy paper, have described the acid-catalysed degradation of (481) obtained by the allylic isomerisation of (480). Four products, (482)–(485), were obtained with toluene-*p*-sulphonic acid in acetic anhydride, but acetic acid in water gave mainly (486) (Scheme 35).[469]

Other Unsaturated Compounds

Appreciable interest has been shown in 2,3-unsaturated cyclic compounds, and in a paper from Lemieux's group a comprehensive account of the synthesis of such derivatives was given and a simple and efficient procedure was recommended. Methyl 2,3-anhydro-4,6-*O*-benzylidene-D-hexopyranosides were treated with sodium iodide in acetone containing sodium acetate and acetic acid, and the resulting iodohydrins were treated with a sulphonyl chloride in refluxing pyridine. In this way the four methyl 4,6-*O*-benzylidene-D-hex-2-enopyranosides were obtained and were found to conform with Hudson's isorotation rule. Details of the n.m.r. spectra were recorded and discussed.[464]

Efforts to prepare branched-chain sugars by application of the known reaction of halides with the cobalt tetracarbonyl anion under carbon monoxide failed, but led to a new method for introducing double bonds into sugar derivatives. With ether as solvent (487) gave (488) in high yield and (489) gave the α-anomer (490) but less readily. When methanol was used as solvent, elimination was accompanied by deacetylation, and the

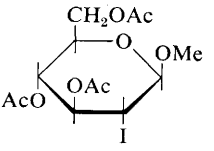
[467] R. J. Ferrier, N. Prasad, and G. H. Sankey, *J. Chem. Soc.* (*C*), 1968, 974.
[468] R. K. Ness and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 181.
[469] M. Katsuhara, S. Wakahara, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1968, **41**, 1208.



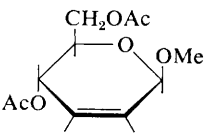
Scheme 35

bromo-analogues of (487) and (489) were unreactive.[470] The rate of solvolytic removal of benzaldehyde from (491) has been measured and was found to be greater than that from a variety of related saturated compounds.[139] Addition of bromine to this olefin was achieved by use of bromine in methanol solution in the presence of barium carbonate and gave the *altro*-dibromide (492) which with base gave (493). Further, (491) with acetyl hypobromite in carbon tetrachloride gave the diequatorial adduct (494) and only small amounts of (495). By carbene addition the adduct (496) was obtained, and was assumed to have the *allo*-configuration.[471] Nitryl iodide did not react with (491) but gave an adduct with a

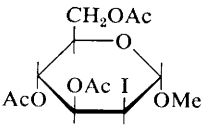
[470] A. Rosenthal and J. N. C. Whyte, *Canad. J. Chem.*, 1968, **46**, 2245.
[471] E. L. Albano, D. Horton, and J. H. Lauterbach, *Chem. Comm.*, 1968, 357.



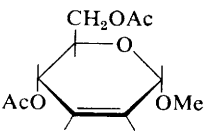
(487)



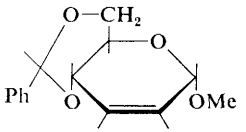
(488)



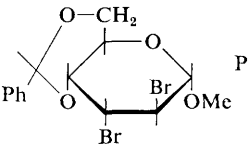
(489)



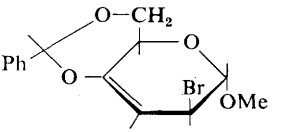
(490)



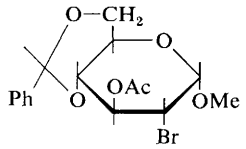
(491)



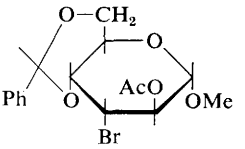
(492)



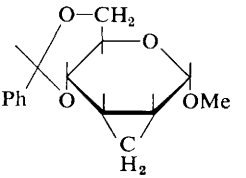
(493)



(494)



(495)

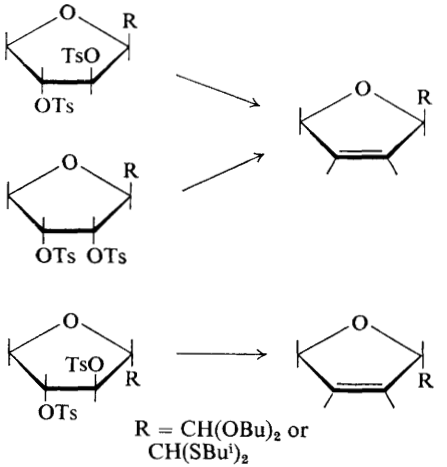


(496)

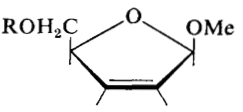
3,4-enoside.[472] Studies have been made on addition reactions to 2-alkoxy-5,6-dideoxy-(2*H*)pyrans as models for unsaturated glycosides.[473]

With five-membered cyclic compounds, elimination of vicinal disulphonyloxy-groups with sodium iodide in DMF in the presence of zinc has been shown to occur with various stereochemical relationships of the ester groups (Scheme 36). Reduction of the products and hydrolysis gave the expected formyl compounds.[474] A full report on compound (497) (Vol. 1, p. 71) has appeared,[475] and the trityl analogue (498) was synthesised by a base-catalysed elimination from a 3-*p*-bromobenzenesulphonyl-2-deoxy-precursor.[476]

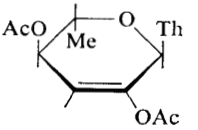
[472] W. A. Szarek, D. G. Lance, and R. L. Beach, *Chem. Comm.*, 1968, 356.
[473] M. Cahu and G. Descotes, *Bull. Soc. chim. France*, 1968, 2975.
[474] J. Defaye, *Bull. Soc. chim. France*, 1968, 2099.
[475] J. Cleophax, J. Hildesheim, and S. D. Gero, *Bull. Soc. chim. France*, 1967, 4111.
[476] N. J. Leonard, F. C. Sciavolino, and V. Nair, *J. Org. Chem.*, 1968, **33**, 3169.



Scheme 36



(497) R = Bz
(498) R = Tr

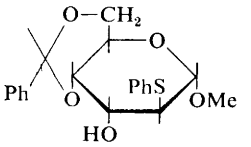


(499)

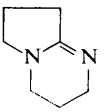
Aspects of the chemistry of several 2,3-unsaturated cyclic compounds bearing substituents on the double bond have been reported. For example, fusion of tetra-*O*-acetyl-L-rhamnopyranose (configuration not specified) with theophylline gave, not the expected rhamnosyl nucleoside, but the unsaturated compound (499).[477] The synthesis of a branched-chain sugar by application of the 'oxo' reaction to a glycosyl acetate structurally related to (499) is referred to on p. 144.

Treatment of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside with sodium thiophenate gave (500) which, after sulphonylation and treatment with 1,5-diazabicyclo[4,5,0]-5-nonene (501), gave the 2-enoside (502) (>90%) which could be desulphurised to give (491). Other elimination conditions also gave (502), but in addition other products including (504) were formed. In the same communication the reaction of (505) with sodium hydride was reported to give (503) together with small amounts of (491) and de-esterified starting material. Compound (506) with potassium t-butoxide in DMSO gave (508) and (491) in the ratio 2 : 1, but the anomer (507) under these conditions gave (491) almost exclusively. The olefin

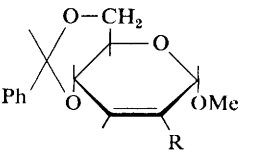
[477] K. Onodera, S. Hirano, F. Masuda, and T. Yajima, *Chem. Comm.*, 1968, 1538.



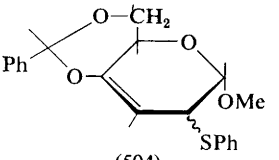
(500)



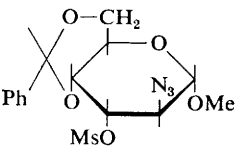
(501)



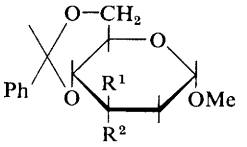
(502) R = SPh
(503) R = N_3



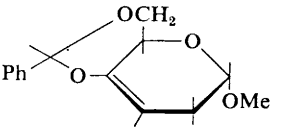
(504)



(505)



(506) R^1 = OMs R^2 = H
(507) R^1 = H R^2 = OMs



(508)

(491) isomerised to (508) with this reagent.[478] Related compounds having nitro-groups attached to C-3 are reported in Chapter 10, and 3,4-unsaturated pyranoid compounds have been obtained from erythromycins (see p. 176).

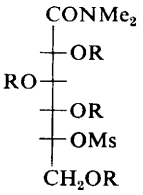
Methyl 4,6-*O*-benzylidene-2,3-di-*O*-methanesulphonyl-α-D-manno- and -allopyranoside on treatment with hot hydrazine undergo elimination of methanesulphonic acid to give enol methanesulphonates which cleave and give the 3-deoxy-2-hydrazone and 2-deoxy-3-hydrazone, respectively.[410]

Treatment of (509) in hot DMF with potassium acetate gave the enol lactone (510) and this with dimethylamine afforded the amide (511). By LAH reduction of this the free sugar (512) was obtained and this could be reconverted to (510) by oxidation. A mechanism for the first reaction involving displacement of the methanesulphonyloxy-group by the carbonyl oxygen atom was proposed.[479]

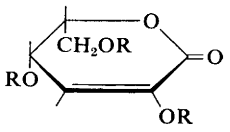
Compound (513) was obtained during the course of studies with 6-cyano-6-deoxy-hexose derivatives.[317b] Compound (514) was produced from the corresponding 3-tosylate. The enone dimerised completely on storage to give a product believed to have structure (515).[480]

The syntheses and reactions of 6-deoxy-hex-5-enoses and 5-deoxy-4-enoses having exocyclic double bonds have been surveyed.[481] New observations have been recorded for each class of compound. The synthesis of the

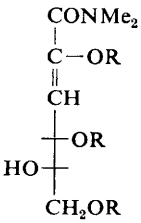
[478] S. Hanessian and N. R. Plessas, *Chem. Comm.*, 1968, 706.
[479] H. Kuzuhara and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 1816.
[480] T. D. Inch and P. Rich, *Carbohydrate Res.*, 1968, **6**, 244.
[481] L. Hough, R. Khan, and B. A. Otter, *Adv. Chem. Ser. No.* 74, 1968, 120.



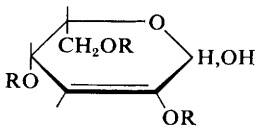
(509)



(510)

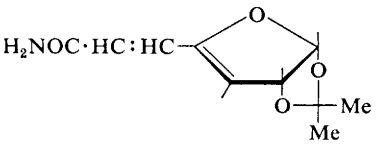


(511)

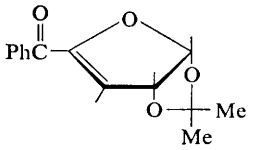


(512)

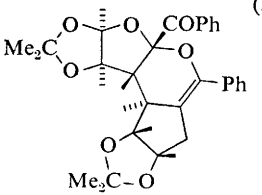
R = CH_2Ph



(513)

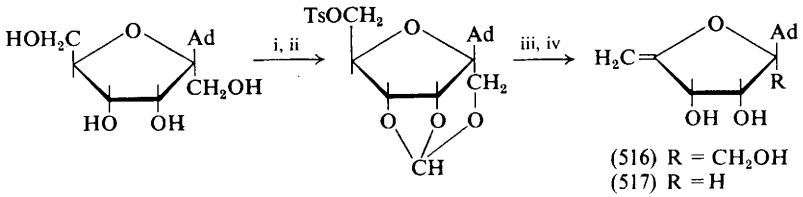


(514)



(515)

4-ene (416) has been described earlier,[433] and the related antibiotic nucleoside angustmycin A (516) has been synthesised from psicofuranine as shown in Scheme 37. Compound (517) was prepared similarly and was converted



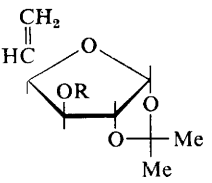
(516) R = CH_2OH
(517) R = H

Reagents: i, $H(COEt)_3$; ii, TsCl–py; iii, $KOBu^t$; iv, H_3O^+

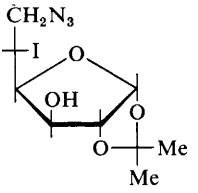
Scheme 37

after bromination to a member of a new class of 3,4′-anhydronucleosides.[482]

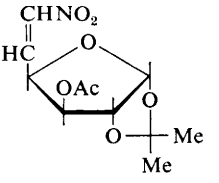
Heating corresponding 5,6-ethoxymethylene derivatives in the presence of an acid catalyst gave (518) in high yield.[483] Addition of iodine azide to the hydroxy-analogue (519) gave the L-*ido*-adduct (521), the structure of



(518) R = Me or CH_2Ph
(519) R = H
(520) R = Ac



(521)

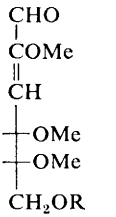


(522)

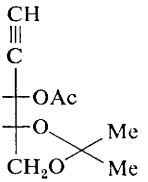
which was established by *X*-ray crystallographic means.[396] Alternatively, nitryl iodide with (520) gave a product which on treatment with sodium bicarbonate gave (522).[472] The adducts obtained by reaction of phosphines with compound (519) are referred to on p. 160.

A new means of obtaining 6-deoxy-hex-5-enosides has been referred to in Chapter 13.[453]

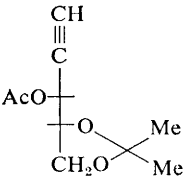
Alkaline treatment of 2,3,4,5-tetra-*O*-methyl-D-glucose gave the *cis*- and *trans*-enol ethers (523),[60] and the *trans*-analogue (524) was obtained



(523) R = H
(524) R = Me



(525)



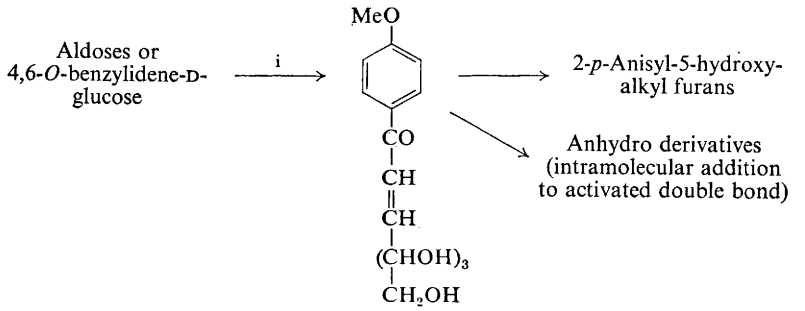
(526)

from the penta-ether. With acid, cleavage of the vinyl ether occurred followed by an elimination β to the new carbonyl group.[484] Other acyclic derivatives have been obtained in an extension of the ethynylation reaction of aldehydo-forms of sugars. From 2,3-*O*-isopropylidene-D-glyceraldehyde, two diastereoisomers were produced which were separated by g.l.c. as their acetates, (525) and (526), which were degraded to D-erythrono- and -D-threono-lactones, and also semihydrogenated to the corresponding

[482] J. R. McCarthy jun., R. K. Robins, and M. J. Robins, *J. Amer. Chem. Soc.*, 1968, **90**, 4993.
[483] J. S. Josan and F. W. Eastwood, *Carbohydrate Res.*, 1968, **7**, 161.
[484] E. F. L. J. Anet, *Carbohydrate Res.*, 1968, **7**, 453.

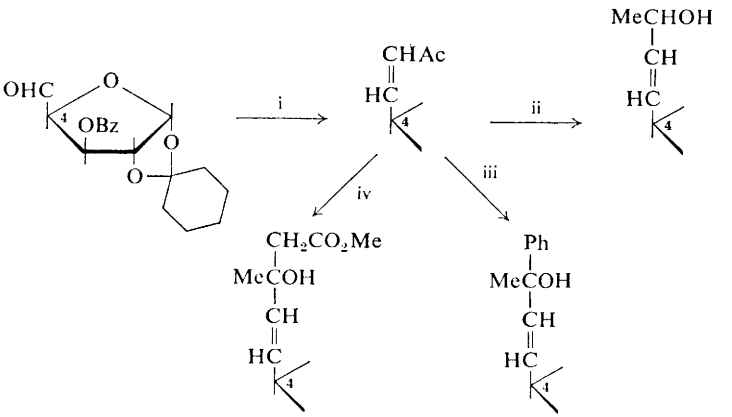
olefins. Esters with the *erythro*-configuration at C-3, C-4 in this series, [*e.g.* (525)], have $J_{3,4}$ *ca.* 4 Hz, whereas the *threo*-isomers have $J_{3,4}$ *ca.* 7 Hz.[485] In analogous fashion vinylation of aldehydo-derivatives followed by ozonolysis provides means for obtaining epimeric aldoses having one carbon added to the starting materials. From 2,3:4,5-di-*O*-isopropylidene-D-arabinose, D-glucose and D-mannose were obtained in the ratio 1·4 : 1, and, similarly, 2,4-*O*-ethylidene-*aldehydo*-D-erythrose gave D-ribose and D-arabinose (4 : 1). The Grignard products obtained in these syntheses were not separated.[486]

Wittig reagents have been applied in reactions with several aldehydic derivatives and gave initially olefinic compounds. These, however, may



Reagent: i, $MeO \cdot C_6H_4 \cdot COCH{=}PPh_3$

Scheme 38



Reagents: i, $Ph_3P{=}CHAc$; ii, $Al(OPr^i)_3$; iii, PhLi; iv, $BrCH_2CO_2Me$

Scheme 39

[485] D. Horton, J. B. Hughes, and J. K. Thomson, *J. Org. Chem.*, 1968, **33**, 728.
[486] D. J. Walton, *Canad. J. Chem.*, 1968, **46**, 3679.

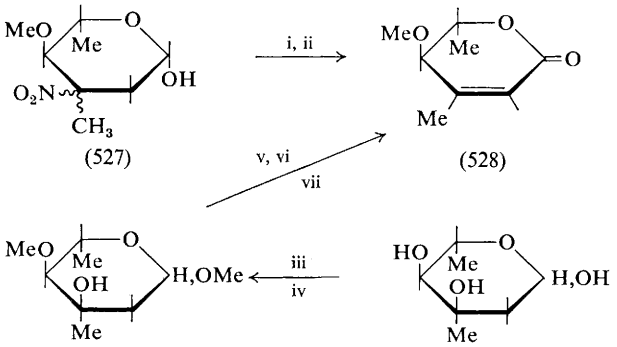
react further or may be converted to non-olefinic final products (2-deoxy-aldoses were thus synthesised [448, 449]). The results are summarised in Schemes 38 [487, 488] and 39.[489]

[487] V. A. Polenov and Yu. A. Zhdanov, *Zhur. obshchei Khim.*, 1967, **37**, 2455.
[488] Yu. A. Zhdanov and V. A. Polenov, *Zhur. obshchei Khim.*, 1968, **38**, 1046.
[489] Yu. A. Zhdanov, Yu. E. Alekseev, and G. N. Dorofeenko, *Zhur. obshchei Khim.*, 1968 **38**, 231.

15
Branched-chain Sugars

A valuable survey of the naturally occurring branched-chain sugars has been made, which included a record of their sources and structures, as well as a discussion of the mechanism of their biosynthesis.[489a]

The most novel report in this area of carbohydrate chemistry is the isolation of a naturally occurring branched-chain nitro-sugar, evernitrose, of partial structure (527), isolated after hydrolysis of everninomicins B and



Reagents: i, Br_2–H_2O; ii, KOAc–MeOH; iii, MeOH–HCl; iv, NaH–MeI; v, H_3O^+; vi, Br_2–H_2O; vii, TsOH

Scheme 40

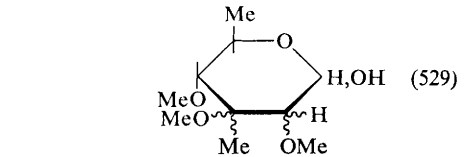
D. The structural evidence was obtained by converting it to (528), which was also synthesised from L-mycarose as shown in Scheme 40.[490] No information is yet available on the configuration at C-3 in (527).

Compounds with an R—C—OR[1] Branch

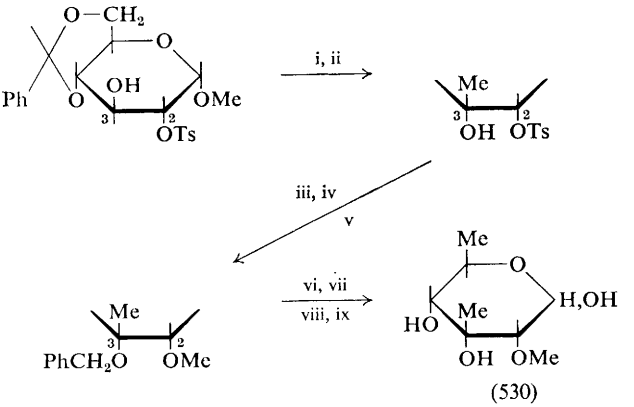
Further examples of naturally-occurring compounds with the Me—C—OR branch at C-3 have received study. Nogalose, a component of the anthracyclinone antibiotic nogalamycin, has been shown to have the partial

[489a] H. Grisebach, *Helv. Chim. Acta*, 1968, **51**, 928.
[490] A. K. Ganguly, O. Z. Sarre, and H. Reimann, *J. Amer. Chem. Soc.*, 1968, **90**, 7129.

structure (529)[491] (see also below). 6-Deoxy-3-*C*-methyl-2-*O*-methyl-D-allose has been synthesised from methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside as shown in Scheme 41.[492] The product



(529)



(530)

Reagents: i, DMSO–DCC–H_3PO_4; ii, MeMgI; iii, $PhCH_2Br$–NaOH; iv, NaOMe; v, Me_2SO_4–NaOH; vi, H_2–Pd; vii, TsCl–py; viii, LAH; ix, H_3O^+

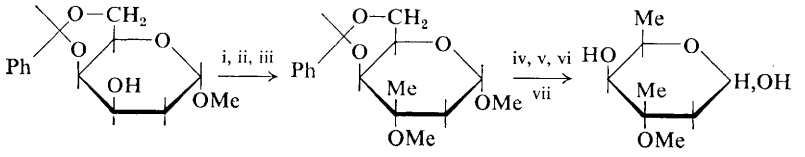
Scheme 41

(530) was shown not to be identical to the branched-chain 6-deoxy-3-*C*-methyl-2-*O*-methyl-L-aldohexose (L-vinelose), the sugar component of a nucleotide derivative (see Vol. 1, p. 137).

Jones and his colleagues have also described the synthesis of arcanose (530a), a component of the antibiotic lankamycin.[493] The synthesis, from methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*lyxo*-hexopyranoside, was accomplished as shown in Scheme 42. The sugar component of olivomycin, olivomycose, has been shown to be (531).[494]

Reaction of methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentosulopyranoside with diazomethane and then LAH gave acetals of the two epimeric

[491] P. F. Wiley, F. A. MacKellar, E. L. Caron, and R. B. Kelly, *Tetrahedron Letters*, 1968, 663.
[492] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1968, **46**, 3375.
[493] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1968, **7**, 284; *Chem. Comm.*, 1968, 62.
[494] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, and V. A. Krivoruchko, *Khim. prirod. Soedinenii.*, 1967, **3**, 405.

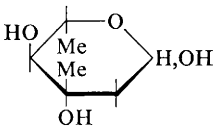


(530a)

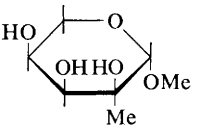
Reagents: i, RuO_4; ii, MeMgI; iii, Me_2SO_4–NaOH; iv, NBS–CCl_4; v, MeONa; vi, LAH; vii, H_3O^+

Scheme 42

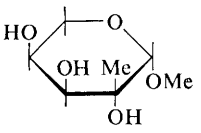
2-*C*-methyl-pentopyranosides, (532) and (533). The major product had the *ribo*-configuration (532).[495] It was shown that the derived branched-chain free sugars were much more stable in both acidic and basic solution than were the corresponding pentoses.



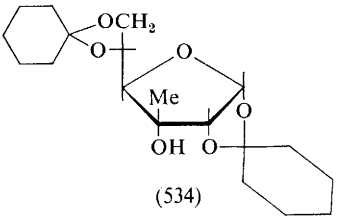
(531)



(532)



(533)



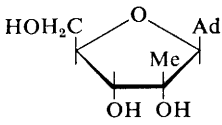
(534)

1,2:5,6-Di-*O*-cyclohexylidene-α-D-*ribo*-hexofuranos-3-ulose when treated with either methyl-lithium or methyl-magnesium bromide gave the same *C*-methyl derivative, (534), with the *allo*-configuration.[496] Reaction of the 3-ulose with phenyl-lithium or with ethyl-, phenyl-, vinyl-, or 1-naphthyl-magnesium halides gave products that, from their n.m.r. spectra, were inferred also to have the *allo*-configuration, as expected from the more likely attack from the β-face of the molecule.

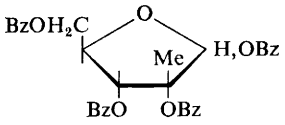
Syntheses of nucleosides having branched-chain sugars of this type have also been reported. 2′-*O*-Methyl-adenosine (535) has been synthesised from α-glucosaccharinic acid lactone, by benzoylation, reduction with bis(3-methyl-2-butyl)borane, and benzoylation to give (536), which was

[495] R. J. Ferrier, W. G. Overend, G. A. Rafferty, H. M. Wall, and N. R. Williams, *J. Chem. Soc.* (*C*), 1968, 1091.

[496] R. D. Rees, K. James, A. R. Tatchell, and R. H. Williams, *J. Chem. Soc.* (*C*), 1968, 2716.

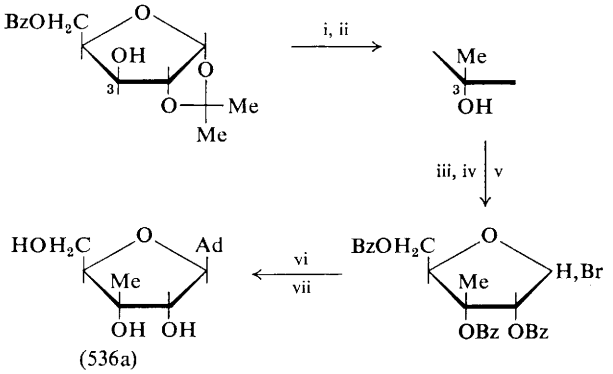


(535)



(536)

converted into the glycosyl chloride and thence into (535) by the mercury method.[497] The same group have also described the synthesis of the isomeric 3′-*C*-methyl-adenosine (536a) from 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose as shown in Scheme 43.[498] Walton's group have



(536a)

Reagents: i, RuO_4; ii, MeMgI; iii, MeOH–HCl; iv, BzCl–py; v, AcBr–AcOH–HBr; vi, chloromercuri-benzamidopurine; vii, MeONa

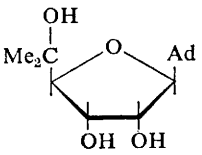
Scheme 43

also described the synthesis of a different type of branched adenosine derivative, namely, (537), which was prepared from the known compound (538), by conversion into the 2,3,5-tri-*O*-benzoyl-glycosyl bromide which was condensed with chloromercuri-6-benzamidopurine.[499]

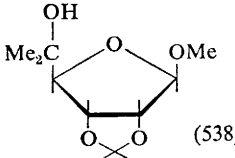
The synthesis of what is believed to be the first synthetic purine nucleoside containing a branched-chain pyranose sugar has been recorded. 6-Deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-D-allopyranose was synthesised and was shown to be different from nogalose. The derived β-acetate was fused with 6-chloropurine in the presence of acid catalysts to give (539).[500]

Another synthesis of the naturally occurring branched-chain pentose, apiose, has been achieved as shown in Scheme 44.[501] Full details[502] have

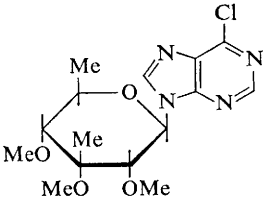
[497] S. R. Jenkins, B. Arison, and E. Walton, *J. Org. Chem.*, 1968, **33**, 2490.
[498] R. F. Nutt, M. J. Dickinson, F. W. Holly, and E. Walton, *J. Org. Chem.*, 1968, **33**, 1789.
[499] R. F. Nutt and E. Walton, *J. Medicin. Chem.*, 1968, **11**, 151.
[500] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1968, **46**, 3691.
[501] J. M. J. Tronchet and J. Tronchet, *Compt. rend.*, 1968, **267**, *C*, 626.
[502] T. D. Inch, R. V. Ley, and P. Rich, *J. Chem. Soc.* (*C*), 1968, 1683.



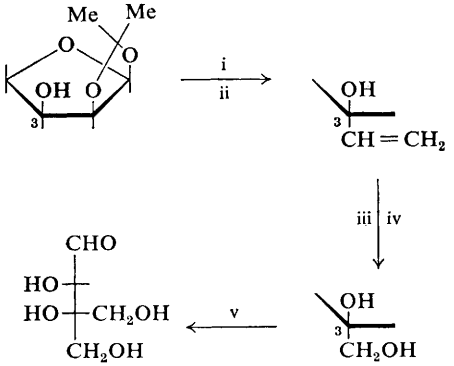
(537)



(538)



(539)



Reagents: i, RuO_4; ii, CH_2:CHMgBr; iii, O_3; iv, LAH; v, H_3O^+

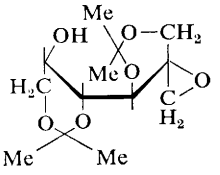
Scheme 44

now been published (*cf.* Vol. 1, p. 136) of the synthesis of 6-deoxy-5-*C*-phenylaldohexose derivatives and their degradation to provide benzylic centres of the type $PhCMe(OH)CO_2H$ of known absolute configuration.[503] The methanolyses of 6-deoxy-5-*C*-phenyl-L-idose and 5(*R*)-5-*C*-cyclohexyl-5-*C*-phenyl-D-xylose were undertaken to investigate the preferred ring-size of these compounds.[59]

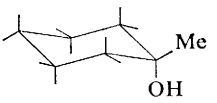
L-Dihydrostreptose, the branched-chain sugar of dihydrostreptomycin, was obtained by sodium amalgam reduction of dihydrostreptosonic acid lactone.[504] The product from the reaction of diazomethane in ether–methanol with 1,3:4,6-di-*O*-isopropylidene-β-L-sorbofuranose has been

[503] T. D. Inch, R. V. Ley, and P. Rich, *J. Chem. Soc.* (*C*), 1968, 1693.
[504] K. Shiroyanagi and H. Ikeda, *Sci. Papers, Inst. Phys. Chem. Res. Tokyo*, 1968, **61**, 150.

shown by n.m.r. to be 2,2′-anhydro-2-*C*-hydroxymethyl-1,3:4,6-di-*O*-isopropylidene-L-*xylo*-hexitol (540).[505]



(540)

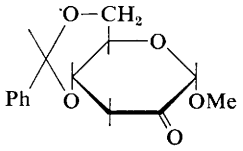


(541)

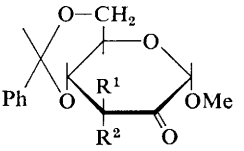
In a paper on 1-methylcyclohexanols it has been pointed out that the conformational free energies of methyl- and hydroxy-groups attached geminally are not additive.[506] Whereas 1-methylcyclohexanol prefers the conformation shown (541) by 0·35 kcal./mole (DMSO) (and near this value in dioxan), the expected value was *ca.* 1·0 kcal./mole. This finding has obvious implication in branch-chain sugar chemistry.

Compounds with an R—C—H Branch

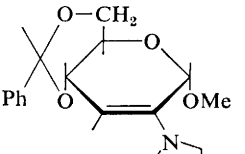
Less work has been reported on this type of branched-chain compound. Treatment of the keto-sugar (542) with barium hydroxide and methyl iodide in DMF, or sodium hydride and methyl iodide in the same solvent, gave the branched-chain sugar (543), also obtained from the enamine (545)



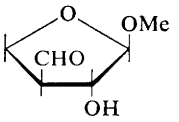
(542)



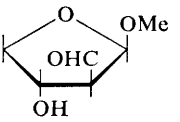
(543) $R^1 = H$, $R^2 = Me$
(544) $R^1 = Me$, $R^2 = H$



(545)



(546)



(546a)

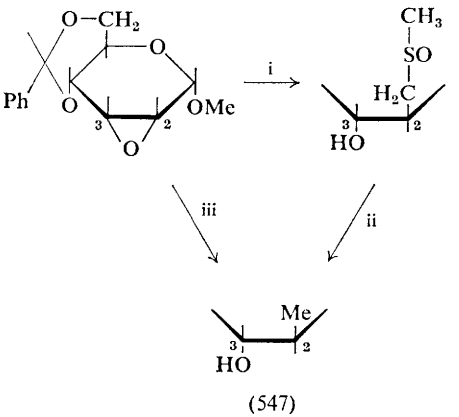
and methyl iodide. The structure of (543) was established by n.m.r. and by its synthesis from methyl 4,6-*O*-benzylidene-3-deoxy-3-*C*-methyl-α-D-altropyranoside by oxidation with ruthenium tetroxide.[507] Compound

[505] T. Maeda, K. Tori, M. Ohtsuru, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1968, **41**, 191.
[506] J. J. Uebel and H. W. Goodwin, *J. Org. Chem.*, 1968, **33**, 3317; N. L. Allinger and C. D. Liang, *J. Org. Chem.*, 1968, **33**, 3319.
[507] R. F. Butterworth, W. G. Overend, and N. R. Williams, *Tetrahedron Letters*, 1968, 3239.

(543) could be epimerised quantitatively into the equatorial methyl compound (544) by treatment with triethylamine in DMF. The above methods have been applied to other carbohydrate derivatives having the CH_2CO group.[507]

Deamination of methyl 3-amino-3-deoxy-β-D-xylopyranoside (*cf.* Vol. 1, p. 135) gave the two branched-chain derivatives (546) and (546a) by ring contraction, the former predominating;[508] both compounds after reduction of the aldehyde group were converted into the corresponding adenine nucleosides.

The branched-chain sugar (547) has been formed by two routes from methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside as shown in



(547)

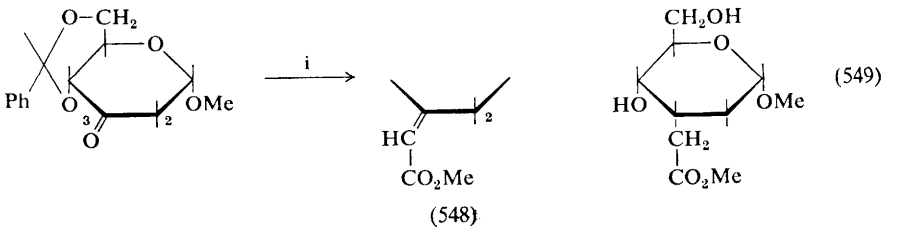
Reagents: i, DMSO–NaH; ii, H_2–Ni; iii, MeLi

Scheme 45

Scheme 45, or by treatment with halide-free methyl-lithium. The *manno*-epoxide with methyl-lithium–lithium iodide gave (79).[171]

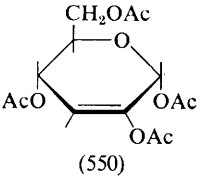
The use of the Wittig reaction on glycosiduloses has been extended[509] to the example shown in Scheme 46. The stereochemistry of the adduct (76% yield) was deduced from the deshielding effect on the 2*e*-H of the carbomethoxymethylene group. Reduction of (548) gave (549) which from its n.m.r. spectrum was assigned the D-*ribo*-configuration. Rosenthal has extended his study of the 'oxo' reaction to the unsaturated sugar (550).[510] The product (551) (after acetylation) was characterised by its n.m.r. spectrum and also by that of the [2-^{2}H]-derivative [synthesised from (550) and CO–D_2]. By-products in the reaction were believed to be (552) and (553). Additions to unsaturated nitro-sugars have given branched-chain compounds.[406] The report of a general synthesis of cyano-sugars

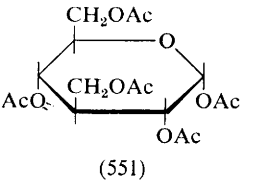
[508] E. J. Reist, D. F. Calkins, and L. Goodman, *J. Amer. Chem. Soc.*, 1968, **90**, 3852.
[509] A. Rosenthal and P. Catsoulacos, *Canad. J. Chem.*, 1968, **46**, 2868.
[510] A. Rosenthal and H. J. Koch, *J. Amer. Chem. Soc.*, 1968, **90**, 2181.

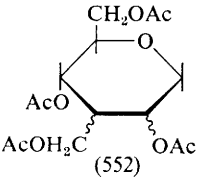


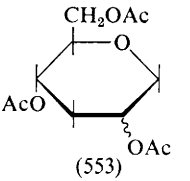
Reagent: i, $(MeO)_2P(O)CH_2CO_2Me$–$KOBu^t$

Scheme 46





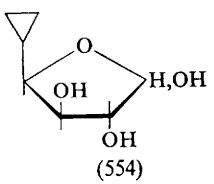


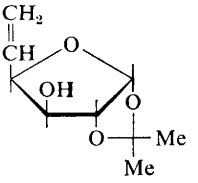


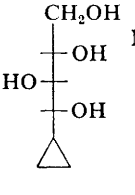
offers a potential route to a wide variety of branched-chain sugars of this class.[412] The first branched-chain sugar derivative with sulphur in the ring has been described.[195]

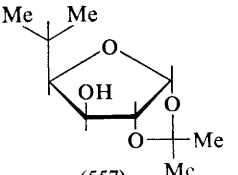
Cyclopropyl Derivatives

Three reports have appeared on this class of derivative. The first deals with compounds with a cyclopropyl side chain.[511] 4-*C*-Cyclopropyl-D-*xylo*-tetrofuranose (554) has been synthesised from the unsaturated sugar (555)







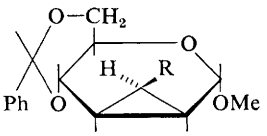


[511] D. Horton and C. G. Tindall jun., *Carbohydrate Res.*, 1968, **8**, 328.

by a methylene insertion reaction (Simmons–Smith method), followed by removal of the blocking groups; sodium borohydride reduction gave the tetritol derivative (556). A variety of experiments showed that the cyclopropyl ring was stable in conditions under which most carbohydrate synthetic work is carried out. Cleavage of the ring by hydrogenolysis in the presence of Raney nickel gave the isopropyl compound (557).

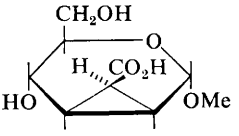
The other reports concerned the synthesis of fused ring systems. Use of the methylene insertion reaction described above on the unsaturated sugar (491) gave a cyclopropyl derivative, which on mechanistic grounds and on the basis of its n.m.r. spectrum was assigned structure (496).[471]

Treatment of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside with ethyl diethoxyphosphoryl acetate [$(EtO_2)P(O)CH_2CO_2Et$] and sodium hydride in dioxan gave, after careful work-up, the acid (558), which was



R

(558) CO_2H
(559) CO_2Me
(560) $CONHNH_2$
(561) CON_3
(562) CH_2OH



(563)

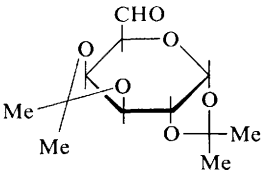
converted into the various derivatives (559)–(562).[512] Hydrolysis of (558) removed the benzylidene ring to give (563), the glycosidic bond of which was stable to 5N-hydrochloric acid.

[512] W. Meyer zu Reckendorf and U. Kamprath-Scholtz, *Angew. Chem. Internat. Edn.*, 1968, 7, 142.

16
Alduloses, Dialdoses, and Diuloses*

Alduloses and diuloses or their derivatives continue to be widely used as intermediates in the synthesis of amino- and branched-chain sugars (see Chapters 8 and 15 respectively). DMSO-based reagents continue to be widely used for their preparation.

The 6-aldehydo-D-galactopyranose derivative (564) has been prepared by Pfitzner–Moffatt oxidation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (the necessity for use of pure samples of the latter compound



(564)

was emphasised). In aqueous solution (564) existed in the aldehydrol form.[513] Photolysis of methyl 6-azido-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (342) gave the 6-aldehydo-derivative (343), characterised as its acetylated aldehydrol (344).[397] Both anomers of methyl 2,3-di-*O*-benzyl-L-*arabino*-pentodialdo-1,4-furanosides (565a) and (565b) have been synthesised as shown in Schemes 47 and 48.[514]

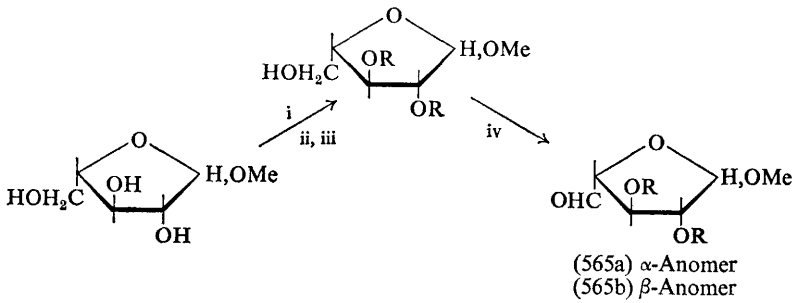
1,2:5,6-Di-*O*-cyclohexylidene-α-D-allofuranose has been prepared by the route previously described for the isopropylidene analogue,[515] namely oxidation of the corresponding glucose compound with DMSO–acetic anhydride, followed by sodium borohydride reduction.

A new route to D-gulose is shown in Scheme 49.[516]

An independent report has appeared on the synthesis of D-allulose derivatives, *via* oxidation of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose and subsequent reduction with complex metal hydrides (*cf.* Vol. 1, p. 138).

[513] D. Horton, M. Nakadate, and J. M. J. Tronchet, *Carbohydrate Res.*, 1968, **7**, 56.
[514] H. Saeki and T. Iwashige, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1129.
[515] M. Kawana, H. Ohrui, and S. Emoto, *Bull. Chem. Soc. Japan*, 1968, **41**, 2199.
[516] G. J. F. Chittenden, *Chem. Comm.*, 1968, 779.

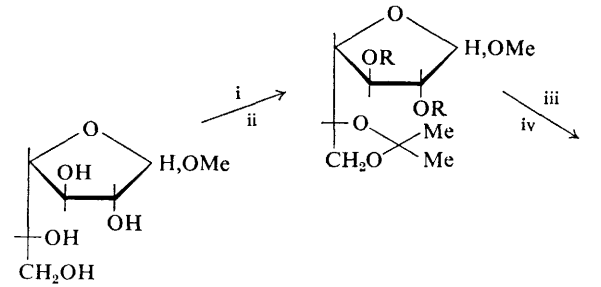
* See also Chapter 22.



(565a) α-Anomer
(565b) β-Anomer

Reagents: i, TrCl–py; ii, $PhCH_2Cl$–KOH–DMSO; iii, aq. AcOH;
iv, DMSO–DCC–H_3PO_4 $R = CH_2Ph$

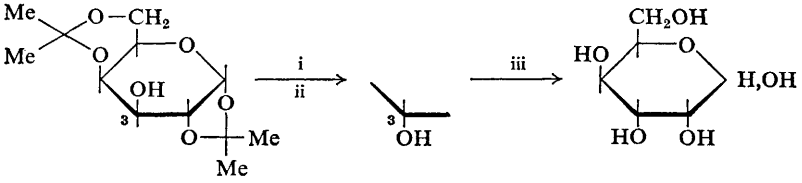
Scheme 47



(565a) and (565b)

Reagents: i, Me_2CO–P_2O_5; ii, $PhCH_2Cl$–KOH–DMSO; iii, aq. AcOH;
iv, $Pb(OAc)_4$–C_6H_6

Scheme 48



Reagents: i, DMSO–Ac_2O or DMSO–P_2O_5; ii, $NaBH_4$; iii, aq. AcOH

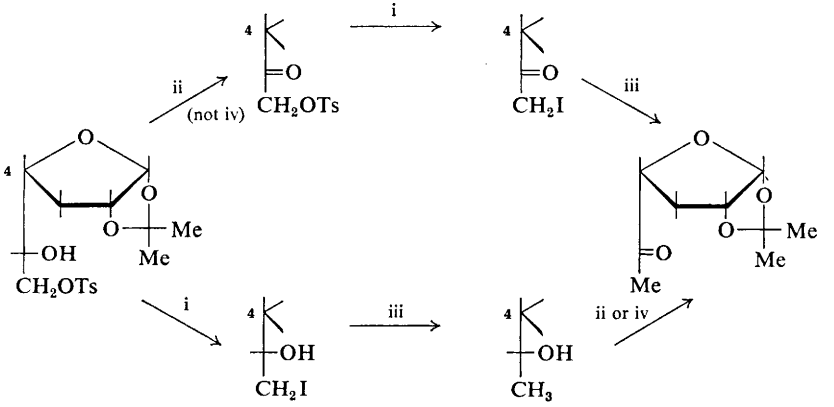
Scheme 49

The present work described the use of ruthenium tetroxide as well as the previously reported DMSO–acetic anhydride.[517]

Several papers have appeared on the synthesis of various aldohex-5-uloses, including the full details of the synthesis of 6-deoxy-D-*arabino*-hexofuranos-5-ulose, shown to be identical with the sugar component of the antibiotic hygromycin A.[518] (The steps in the synthesis were given in

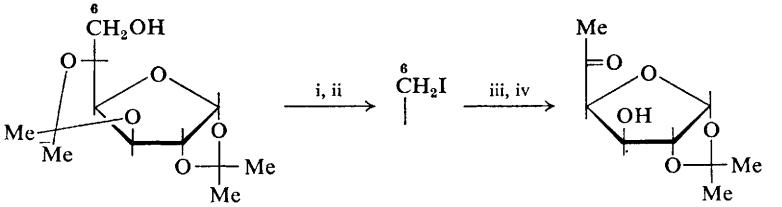
[517] G. M. Cree and A. S. Perlin, *Canad. J. Biochem.*, 1968, **46**, 765.
[518] S. Takahashi and M. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 1082.

Vol. 1, p. 131.) 3,6-Dideoxy-1,2-*O*-isopropylidene-L-*threo*-hexofuranos-5-ulose has been synthesised as shown in Scheme 50.[519] 6-Deoxy-1,2-*O*-isopropylidene-D-*xylo*-hexofuranos-5-ulose has been synthesised by a similar sequence and also by the route in Scheme 51.[520] The synthesis of



Reagents: i, NaI–Me_2CO; ii, DMSO–Ac_2O; iii, Ni–H_2; iv, O_2–Pt

Scheme 50



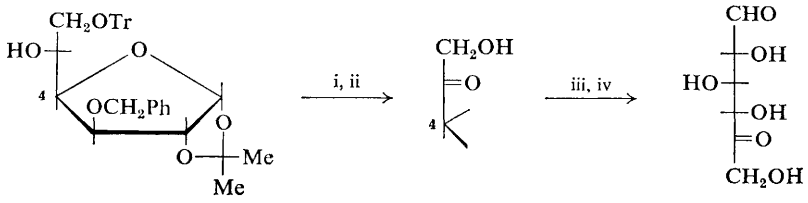
Reagents: i, TsCl–py; ii, NaI–Me_2CO; iii, AgF–py; iv, H_3O^+

Scheme 51

D-*xylo*-hexos-5-ulose 6-phosphate has been reported.[241] The product resulting from the treatment of 1,2-*O*-isopropylidene-5-*C*-phenyl-3-*O*-toluene-*p*-sulphonyl-α-D-xylopentofuranos-5-ulose with sodium carbonate in DMF has already been described.[480]

D-*xylo*-Hexos-5-ulose (566) has been shown to cyclise readily in alkaline solution to give *myo*-inosose-2;[521] compound (566) was prepared by the route shown in Scheme 52.

[519] K. Antonakis, *Bull. Soc. chim., France*, 1968, 2972.
[520] M. Nakajima and S. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 1079.
[521] D. E. Kiely and H. G. Fletcher jun., *J. Amer. Chem. Soc.*, 1968, **90**, 3289.

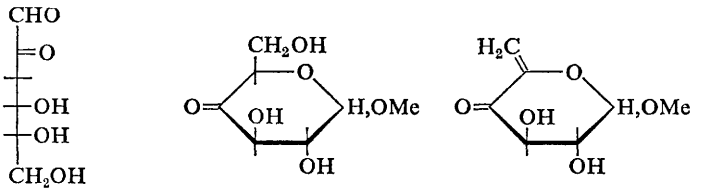


(566)

Reagents: i, DMSO–Ac_2O; ii, H_3O^+; iii, H_2–Pd; iv, H_3O^+

Scheme 52

It has been shown that 3-deoxy-D-*erythro*-hexos-2-ulose ('3-deoxy-gluculose') (567) is produced on the degradation of difructosylglycine,[522] but the mechanism proposed has been refuted, since it was claimed [523] that the i.r., and in particular the n.m.r. evidence were based on incorrect assignments. Compound (567) has also been of recent interest because of its suspected activity as a regulator of mitosis (although it has since been found to be physiologically inactive and not to occur in the free form); it was therefore treated with several thiol and amino-compounds, and found to be much less reactive than typical α-keto-aldehydes. This low reactivity was attributed to the existence of (567) in the pyranose-ring form, and its stability was believed to make the likelihood of its activity as a mitotic regulator small.[523a] The proposal that D-glucosone was an intermediate in the oxidation of D-glucose with sodium polysulphide has found support.[524]

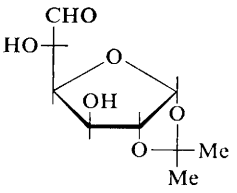


(567) (568) (569)

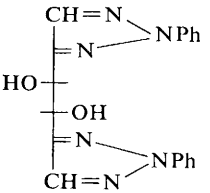
A study has been made of model reactions for the enzymic reactions that are alleged to proceed *via* aldohex-4-ulose intermediates.[525] Catalytic oxidation of methyl α-D-galactopyranoside with platinum and oxygen, followed by hydrogenation, gave methyl α-D-fucopyranoside (15%); for the β-series the yield was 35%. It was proposed that the initially formed oxidation product (568) was unstable, and eliminated water to yield the hexulosid-5,6-ene (569), which was reduced stereospecifically.

[522] G. Fodor and J.-P. Sachetto, *Tetrahedron Letters*, 1968, 401.
[523] E. F. L. J. Anet, *Tetrahedron Letters*, 1968, 3525.
[523a] E. Jellum, *Acta Chem. Scand.*, 1968, **22**, 1722.
[524] B. Lindberg and O. Theander, *Acta Chem. Scand.*, 1968, **22**, 1782.
[525] O. Gabriel, *Carbohydrate Res.*, 1968, **6**, 111.

Two papers have appeared on the production of carbonyl compounds using DMSO–boron trifluoride. Oxidation of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose gave the aldehydo-sugar (570), which

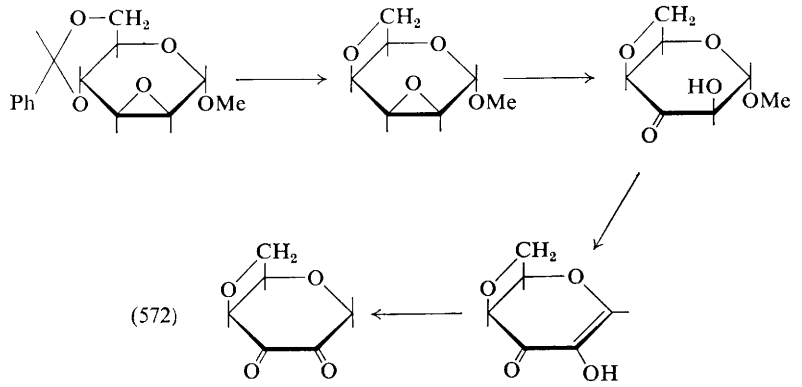


(570)



(571)

formed a phenylhydrazone, and a 5,6-phenylosazone which could be oxidised to a phenylosotriazole. A corresponding series of reactions was carried out on 5,6-anhydro-D-*arabino*-phenylosotriazole, and the product eventually converted into the bisosotriazole (571).[526] Treatment of methyl 2,3-anhydro-α-D-allo- or manno-pyranoside or their 4,6-*O*-benzylidene acetals with DMSO–boron trifluoride gave compound (572), which was believed to be formed, for example, as in Scheme 53.[527] Several bishydrazine



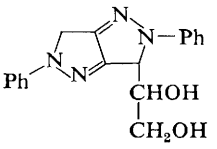
(572)

Scheme 53

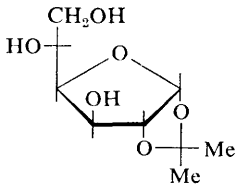
derivatives were prepared; the phenylhydrazone on treatment with cupric sulphate gave compound (573).

Radiolysis of methyl α-D-glucopyranoside has been shown to give the 5-deoxy-5-ketohexose (54)[114] and the aldehydo-derivative (55).[115] The

[526] G. Hanisch and G. Henseke, *Chem. Ber.*, 1968, **101**, 2074.
[527] G. Hanisch and G. Henseke, *Chem. Ber.*, 1968, **101**, 4170.



(573)



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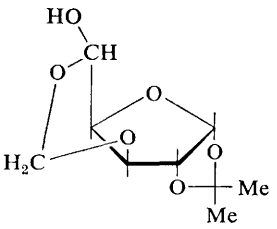

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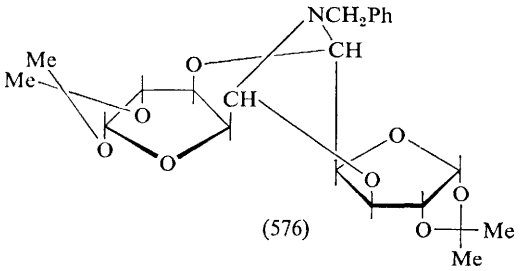

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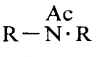

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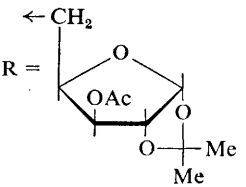
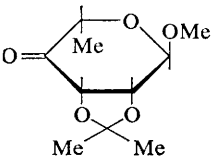


product of periodate oxidation of (574) has been shown to exist as a mixture of a dimer, linked through acetal bonds, and the methylidene compound (575). Reaction of the product with benzylamine gave the dimer derivative (576). On hydrogenation in neutral solution this underwent debenzylation, but in acid solution gave (after acetylation) (577).[528]

It has been observed that D-*threo*-hex-2,5-diulose was converted into a mixture of kojic acid and 5-oxymaltol on heating in aqueous solution under pressure. The ratio of products varied from 2·8 : 1 to 6·1 : 1 depending on the conditions used. Another unidentified γ-pyrone derivative was also formed in the reaction.[529]

[528] H. Paulsen and K. Todt, *Chem. Ber.*, 1968, **101**, 3358.
[529] S. Oga, K. Imada, K. Asano, K. Aida, and T. Uemura, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 1511.

Irradiation of the keto-sugar (578) caused[530] elimination of carbon monoxide and ring contraction to the furanoside derivatives (579) and (580),



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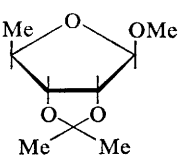

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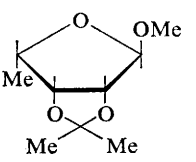


(580)

formed in the ratio 9 : 1. Radiolysis of cellobiose and lactose in the absence of oxygen gave, amongst other products, 2-deoxy-hexos-3-uloses.[451]

[530] P. M. Collins, *Chem. Comm.*, 1968, 403.

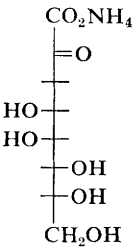
17
Sugar Acids and Lactones

Aldonic Acids

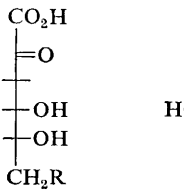
An interesting g.l.c. method has been developed for the determination of the configuration of aldonic acids at C-2, following the observation that the diastereoisomeric products of esterification of resolvable alcohols with acetylated aldonyl chlorides are separable on capillary columns; the best separations were achieved with sterically crowded alcohols, for example 3,3-dimethylbutan-2-ol. When the C-2 configuration of the acid was D, the ester formed from the (+)-alcohol was eluted first. As a corollary, if the relative configuration of the acid is known, the absolute configuration follows. Under the conditions used the hexose derivatives were too involatile, but it was observed that these could be degraded specifically before application of the procedure.[531]

The synthesis of benzylidene acetals of pentonic acids was achieved by oxidation of the *aldehydo*-analogues with chromic oxide in DMF. Preparation of the same type of derivative from pentonic acid salts or esters, with benzaldehyde and acid catalysts, gave dibenzylidene acetals, and monoacetals of the acids and lactones.[532]

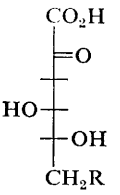
Two papers have appeared on the synthesis of ammonium 3-deoxy-D-*manno*-octulosonate (581) from D-arabinose and oxalacetates. In one procedure, the *gluco*-isomer was also obtained.[533] In a related study, the



(581)



(582) R = H
(583) R = OH



[531] G. E. Pollock and D. A. Jermany, *J. Gas Chromatog.*, 1968, **6**, 412.
[532] H. Zinner, H. Voigt, and J. Voigt, *Carbohydrate Res.*, 1968, **7**, 38.
[533] C. Hershberger and S. B. Binkley, *J. Biol. Chem.*, 1968, **243**, 1578; C. Hershberger, M. Davis, and S. B. Binkley, *J. Biol. Chem.*, 1968, **243**, 1585.

synthesis of the epimeric 3,6-dideoxy-D-hexulosonic acids (582) was achieved by condensing oxalacetic acid with lactaldehyde; the 3-hydroxy-analogues (583) were obtained similarly from D (or L)-glyceraldehyde. When R = OH, the *erythro* : *threo* ratio of the products was 2 : 1, but with R = H it was 10 : 1. These results were discussed in terms of Cram's rule.[534]

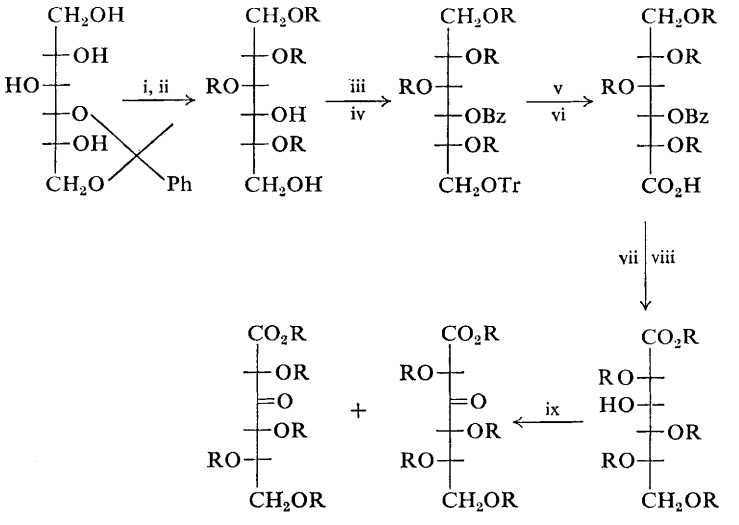
It has been proposed that D-glucosone is the primary oxidation product of treatment of D-glucose with sodium polysulphide, and that this then undergoes alkaline degradation to a mixture of carboxylic acids. Strong support is provided for this proposal by the finding of the same acids in the products of treatment of D-glucosone with alkali. Calcium hydroxide promoted a benzilic acid rearrangement to give D-mannonic acid as the main product, whereas sodium hydroxide caused cleavage of the C-(1)–C-(2) bond and resulted chiefly in D-arabinonic acid. Other acidic products (notably gluconic, ribonic, and erythronic acids) were produced in small amounts. These were analysed by the g.l.c. of TMS derivatives of the lactones.[524]

D-Galactose and its 3-deoxy-derivative on oxidation with *Pseudomonas putida* gave the aldonic acids, which then reacted further to give the 2-keto-compounds.[535] The enzyme responsible was purified and characterised.[536] Catalytic hydrogenation of the related calcium D-*xylo*-hex-5-ulosonate gave the D-gluconate and L-idonate which were shown to be readily separable by g.l.c. after lactonisation and formation of the TMS ethers.[537] The epimeric D-*lyxo*-hex-5-ulosonic acid has been obtained in 60–70% yield by base-catalysed isomerisation of D-glucurono-γ-lactone and may also be prepared by bromine oxidation of L-gulono-γ-lactone.[538]

Benzyl 2,4,5,6-tetra-*O*-benzyl-L-gulonate (584), a potential precursor of 3-keto-L-gulonic acid, has been synthesised as illustrated in Scheme 54. Debenzylation of the mixed keto-esters gave (585) and (586), illustrating the instability of β-keto-esters.[539]

Lactone (587) has been epimerised to (588), and the isomers were separated and reduced to give 1,3-dideoxy-D-*ribo*- and L-*lyxo*-hexitol, respectively.[540] The partial reduction of acylated aldono-γ-lactones to acylated furanoses was found to occur most satisfactorily with freshly prepared bis-(3-methyl-2-butyl)borane.[541] 2,3:5,6-Di-*O*-isopropylidene-D-gulono-γ-lactone was reduced with sodium borohydride to the free sugar

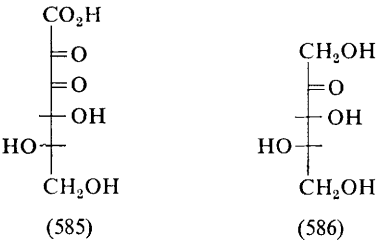
[534] D. Portsmouth, *Carbohydrate Res.*, 1968, **8**, 193.
[535] H. W. Schiwara and G. F. Domagk, *Z. Physiol. Chem.*, 1968, **349**, 297.
[536] H. W. Schiwara and G. F. Domagk, *Z. Physiol. Chem.*, 1968, **349**, 1321.
[537] C.-C. Chen, T. Imanari, H. Yamamoto, and T. Kwan, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 755.
[538] E. R. Nelson and P. F. Nelson, *Austral. J. Chem.*, 1968, **21**, 2323.
[539] M. Matsui, M. Saito, M. Okada, and M. Ishidate, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1294.
[540] J. Němec, Z. Kefurtová, K. Kefurt, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1968, **33**, 2097.
[541] P. Kohn, L. M. Lerner, A. Chan jun., S. D. Ginocchio, and C. A. Zitrin, *Carbohydrate Res.*, 1968, **7**, 21.



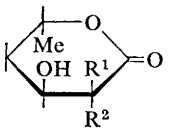
(584)

Reagents: i, $PhCH_2Cl$–KOH; ii, EtOH–H_3O^+; iii, TrCl–py; iv, BzCl–py; v, H_3O^+; vi, CrO_3–py; vii, MeO^-; viii, $PhCH_2OH$–DCC; ix, DMSO–DCC–H_3PO_4

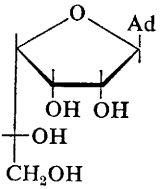
Scheme 54 R = CH_2Ph



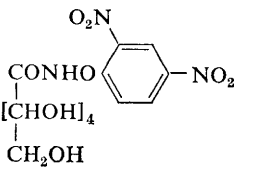
(585) (586)



(587) R^1 = OH, R^2 = H
(588) R^1 = H, R^2 = OH



(589)



(590)

which was converted to the glycosyl chloride and used in the synthesis of the gulopyranosyl nucleoside (589).[542]

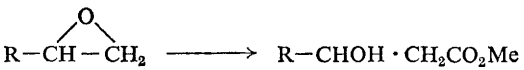
[542] L. M. Lerner, B. D. Kohn, and P. Kohn, *J. Org. Chem.*, 1968, **33**, 1780.

The synthesis of a series of *O*-acylaldonamides of the pentoses, hexoses, and heptoses has been carried out by hydrolysis of the appropriate nitriles,[543] and the formation of unsaturated products from a gluconamide sulphonate has already been described.[479] D-Glucono-1,5-lactone with hydroxylamine in basic solution gave the hydroxamate which with fluoro-2,4-dinitrobenzene gave (590). This on heating gave gluconic acid together with D-arabinose, but since the yield of the latter was only 7%, the reactions are not yet suitable for removing C-1 of aldonic acids in preparations of lower aldoses.[544]

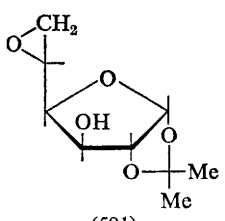
Mention is made of various metal gluconates in Chapter 18.

Uronic Acids

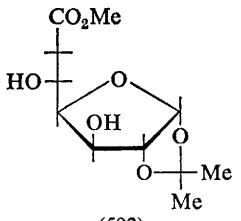
The reaction of sugar epoxides with sodium cobalt tetracarbonyl and carbon monoxide in methanol was assessed as a possible route to branched-chain sugars since the reaction in Scheme 55 is well established. Compound (591) conformed with expectations to give (592) in good yield in a new synthesis of uronic acid derivatives; the ketone (593), however, was also



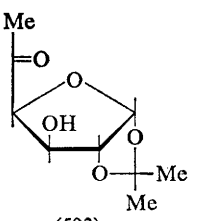
Scheme 55



(591)



(592)



(593)

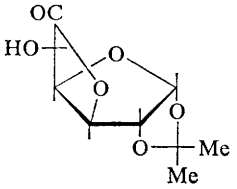
produced. The reaction did not prove to be of general applicability since the only effect on methyl 5-*O*-acetyl-2,3-anhydro-β-D-ribofuranoside was deacetylation and 3,4,6-tri-*O*-acetyl-1,2-anhydro-D-glucose afforded tri-*O*-acetyl-D-glucal. Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside rearranged partially to a ketose and in part underwent methanolysis.[545]

The o.r.d. curves of methyl glycosides of uronic acids have been measured (see p. 204), and methylated derivatives have been examined by mass spectrometry (see p. 199).

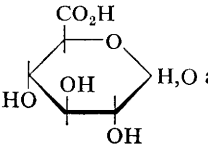
A series of selectively acylated derivatives of D-glucuronic acid have been synthesised from (594) and the corresponding methyl β-glycoside. The

[543] J. Deferarri and B. Matsuhiro, *Anales Asoc. quím. argentina*, 1967, **55**, 187 (*Chem. Abs.*, 1968, **69**, 67645g).
[544] J. N. BeMiller and G. T. Wisely, *Trans. Illinois State Acad. Sci.*, 1967, **60**, 117.
[545] A. Rosenthal and J. N. C. Whyte, *Canad. J. Chem.*, 1968, **46**, 2239.

three monosulphonyl derivatives of methyl β-D-glucofururonic acid amide and nitrile and related esters derived from (594) were also prepared in this investigation as potential precursors of amino-sugar derivatives.[546]



(594)



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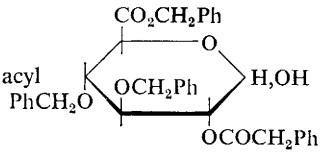


(596)

Methods have been described for the preparation of the hitherto inaccessible 1-*O*-acyl-D-glucopyranuronic acids (595) *via* (596). 1-*O*-Aroyl derivatives were described with α- and β-configurations, the acyl halide in pyridine giving predominantly α-anomers, while reactions involving the glycosyl bromides as intermediates provided a means for obtaining the β-isomers.[547] Synthetic 3-amino-3-deoxy-D-alluronic acid has been described.[319]

Reference is made to the hydrolysis of uronosides in Chapter 3, and in other studies connected with polysaccharide chemistry, 6-*O*-α-D-glucopyranuronosyl-D-glucose was synthesised as its acetylated methyl ester by standard procedures from β-isomaltose octa-acetate, and improvements were reported in the preparation of the corresponding derivatives of 6-*O*-β- and 4-*O*-α-D-glucopyranuronosyl-D-glucose.[548] Further, a series of wood-pulp polysaccharide hydrolysates were shown to contain 4-*O*-methyl-L-iduronic acid formed during alkali treatment of the commonly occurring D-*gluco*-isomer. Analysis was carried out by a combination of physical and chemical procedures, the position of the methyl group being assigned by mass spectrometry of the TMS derivative.[549]

Uronic acid lactones have been utilised in the synthesis of hex-5-ulosonic acids,[538] and the reaction of *p*-nitrophenyl glucuronoside with oxidising agents gave glucuronic acid and glucaric acid. Under the same conditions glucuronic acid itself gave glucaric acid.[550]

Hexopyranuronates are subject to a reverse anomeric effect operating at C-5 (see p. 192).

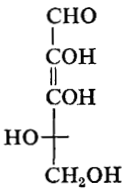
Ascorbic Acid and Related Enediols

A synthesis of ascorbic acid from L-sorbose has been described,[551] and the kinetics of oxidation of the acid by the vanadyl ion have been investigated

[546] H. Weidmann, D. Wewerka, and N. Wolf, *Monatsh.*, 1968, **99**, 509.
[547] D. Keglevíc, N. Pravdić, and J. Tomašić, *J. Chem. Soc.* (*C*), 1968, 511.
[548] N. Roy and C. P. J. Glaudemans, *J. Org. Chem.*, 1968, **33**, 1559.
[549] E. R. Nelson, P. F. Nelson, and O. Samuelson, *Acta Chem. Scand.*, 1968, **22**, 691.
[550] Y. Yamane, M. Miyazaki, and K. Sakai, *J. Pharm. Soc. Japan*, 1968, **88**, 191.
[551] J. M. Romero, *Anales Farm. Hosp.*, 1967, **10**, 127 (*Chem. Abs.*, 1968, **68**, 13298).

in detail, and mechanisms for the reaction have been discussed.[552] The crystal structure has been examined (see p. 201), as have the c.d. curves at different pH values (see p. 205).

The osazone formed from dehydro-L-ascorbic acid has been shown to have a six-membered ring,[368] and some of its reactions have been described.[371] Similar attention has been given to related compounds.[369, 370, 372] Non-oxidative degradation of the same dehydroascorbic acid gave the *aci*-reductone (597) by decarboxylation.[553]



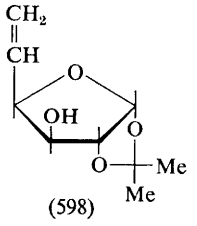
(597)

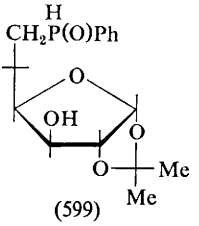
[552] M. M. Taqui Khan and A. E. Martell, *J. Amer. Chem. Soc.*, 1968, **90**, 6011.
[553] K. Wisser, W. Heimann, and E. Mögel, *Angew. Chem. Internat. Edn.*, 1967, **7**, 732.

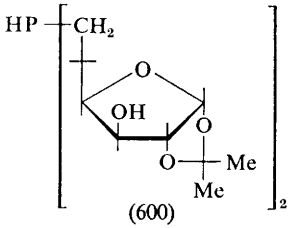
18
Inorganic Derivatives

Carbon-bonded Compounds

Photochemical addition of phenylphosphine to compound (598) gave a disubstituted phosphine isolated in high yield as its oxide (599). Phosphine


(598)


(599)


(600)

itself apparently gave a corresponding simple adduct together with the diglycosyl phosphine (600) both of which were obtained as oxidised products.[554]

Nucleoside derivatives related to compound (599) have been prepared by different means involving application of Wittig reactions.[545a]

An improvement in the partial synthesis of 5′-deoxyadenosylcobalamin is reported by use of 2′,3′-*O*-*p*-anisylidene-5′-*O*-toluene-*p*-sulphonyl-adenosine and hydroxocobalamin. The acetal ring was removed from the condensed product with acid conditions sufficiently mild to give 85% of the pure product. This represents an improvement on the isopropylidene method by which undesired by-products are obtained at the hydrolysis step.[555]

Oxygen-bonded Compounds

The chelation constants for the reaction between arsenious acid and fructose (1 : 1 complex formed) have been reported, and thermodynamic parameters have been calculated.[556]

[554] R. L. Whistler, C.-C. Wang, and S. Inokawa, *J. Org. Chem.*, 1968, **33**, 2495.
[554a] G. H. Jones and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1968, **90**, 5337.
[555] C. G. D. Morley and H. P. C. Hogenkamp, *Arch. Biochem. Biophys.*, 1968, **123**, 207.
[556] P. J. Antikainen and I. P. Pitkanen, *Suomen Kem.*, 1968, **41**, 108.

Polymeric structures were proposed to account for the i.r. spectral, visible reflectance spectral, and the magnetic and the thermal decomposition characteristics of nickel gluconate $Ni(C_6H_{11}O_7)_2,2H_2O$ and nickel hydroxygluconate $Ni(OH)_2(C_6H_{10}O_7),2H_2O$.[557] The properties of the simple gluconates of manganese, iron, zinc, cadmium, barium, and lead (all II) and related complexes were examined by various methods.[558]

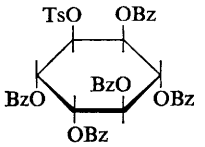
[557] G. A. Melson and W. F. Pickering, *Austral. J. Chem.*, 1968, **21**, 1205.
[558] G. A. Melson and W. F. Pickering, *Austral. J. Chem.*, 1968, **21**, 2889.

19
Cyclitols

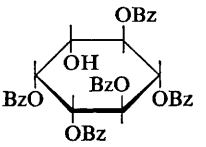
A much needed set of rules for cyclitol nomenclature has been published jointly by the IUPAC commission on the nomenclature of organic chemistry and the IUPAC–IUB commission on biochemical nomenclature.[559] A review on inositol chemistry has appeared that discussed their isomerism, physical properties, synthesis, and derivatives, including inosamines.[559a]

myo-Inosose-2 and *scyllo*-inositol have been identified as constituents of rat sciatic nerve and of calf brain; the compounds were isolated by g.l.c. of their TMS derivatives.[560] The latter derivative and D-glycero-1-(L-*myo*-inositol 1-hydrogen phosphate) have been found to occur in the male reproductive tract, with the previously isolated *myo*-inositol.[561] An inositol derivative isolated from a species of juniper has been tentatively characterised as a methyl ether of *muco*-inositol.[562] Inositol phosphates have been obtained from the phosphatido-peptide fraction of pig aorta.[563] The chemistry of deoxy-cyclitols, with particular reference to (+)-*proto*-quercitol, has been reviewed,[564] and full details of the synthesis of the latter compound from (−)-inositol have been described[565] (see Vol. 1, p. 152).

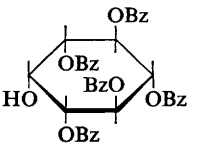
Treatment of 2,3,4,5,6-penta-*O*-benzoyl-1-*O*-toluene-*p*-sulphonyl-(−)-inositol (601) with sodium fluoride in DMF at 140° gave two products 1,2,4,5,6- and 1,3,4,5,6-penta-*O*-benzoyl-*myo*-inositol (602) and (603),



(601)



(602)

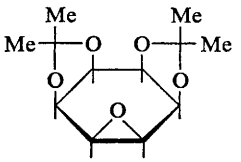


(603)

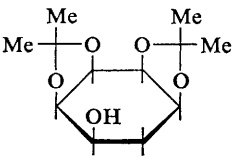
[559] *Arch. Biochem. Biophys.*, 1969, **128**, 269; *European J. Biochem.*, 1968, **5**, 1.
[559a] G. Kimura and E. Noda, *J. Synthetic Org. Chem., Japan*, 1967, **25**, 764.
[560] W. R. Sherman, M. A. Stewart, P. C. Simpson, and S. L. Goodwin, *Biochemistry*, 1968, **7**, 819.
[561] R. F. Seamark, M. E. Tate, and T. C. Smeaton, *J. Biol. Chem.*, 1968, **243**, 2424.
[562] L. M. Utkin, *Khim. prirod. Soedinenii*, 1968, 277.
[563] M. Hierowski, *Z. Physiol. Chem.*, 1968, **349**, 357.
[564] G. E. McCasland, M. O. Naumann, and S. Furuta, *Adv. Chem. Ser. No.* 74, 1968, 41.
[565] G. E. McCasland, M. O. Naumann, and L. J. Durham, *J. Org. Chem.*, 1968, **33**, 4220.

respectively,[566] which were separated by p.l.c. of their pyranyl derivatives and structures of which were proved by debenzoylation. Selective benzoylation of 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol gave the 3-ester, which was then phosphorylated and the blocking groups were removed to give the 4-phosphate as its cyclohexylammonium salt.[567] A method for the separation of the four possible pentaphosphates of *myo*-inositol, based on moving-paper electrophoresis, has been described.[568]

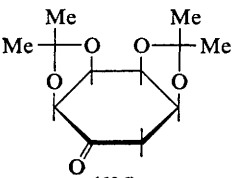
1,2-Anhydro-3,4:5,6-di-*O*-isopropylidene-*cis*-inositol (604) when reduced with LAH gave (±)-6-deoxy-1,2:3,4-di-*O*-isopropylidene-*cis*-inositol (605), oxidation of which with the Pfitzner–Moffatt reagent gave the corresponding



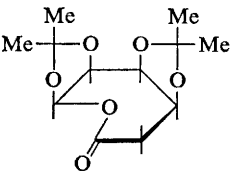
(604)



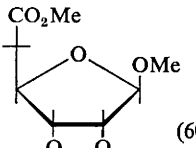
(605)



(606)



(607)

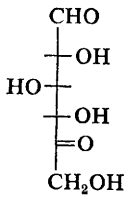


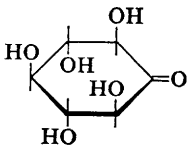
(608)

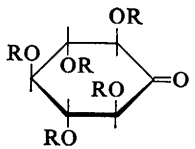
keto-compound (606);[569] manganese dioxide also gave the normal oxidation product (606) and not the oxygen insertion product observed with (+)-1,2:3,4-di-*O*-isopropylidene-6-*O*-methyl-*epi*-inositol (Vol. 1, p. 155). Baeyer–Villiger oxidation of (606) gave the hemi-acetal lactone, believed to have structure (607) and not the alternative possibility. LAH reduction of (607) gave 2-deoxy-3,4-*O*-isopropylidene-(±)-allitol, which on periodate oxidation and acid hydrolysis gave 4-deoxy-(±)-D-ribose. Treatment of (607) with methanolic sulphuric acid gave a product believed to be methyl [methyl 5-deoxy-2,3-*O*-isopropylidene-β-(±)-allofuranoside] uronate (608), characterised by LAH reduction to methyl 5-deoxy-2,3-*O*-isopropylidene-β-(±)-allofuranoside, that was further converted into the 5,6-dideoxy-derivative. The (±) compounds were identified by comparison with the known D-isomers.[569]

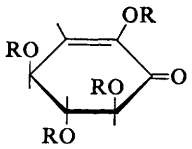
It has been demonstrated that D-*xylo*-hexos-5-ulose (609) cyclised readily in alkaline solution to *myo*-inosose-2 (610), which was characterised by its

[566] D. Mercier and S. D. Gero, *Tetrahedron Letters*, 1968, 3459.
[567] N. B. Tarusova, V. S. Grosheva, S. P. Kozlova, R. B. Teplinskaya, and N. A. Preobrazhenskii, *Zhur. org. Khim.*, 1968, **4**, 967.
[568] M. E. Tate, *Analyt. Biochem.*, 1968, **23**, 141.
[569] H. Fukami, H.-S. Koh, T. Sakata, and M. Nakajima, *Tetrahedron Letters*, 1968, 1701.

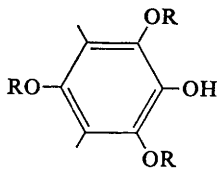

(609)


(610)


(611)

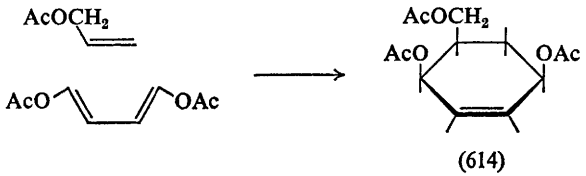

(612)

R = CH_2Ph


(613)

reduction to a mixture of *scyllo*- and *myo*-inositol.[521] It was suggested that this type of pathway could account for the biosynthesis of *myo*-inositol from D-glucose and its 6-phosphate. The inosose ether (611), prepared from *myo*-inositol, on treatment with $Ph_3P{:}CHCO_2H$ underwent elimination yielding (612), rather than the expected Wittig adduct. Reaction of (611) with potassium t-butoxide in DMF caused aromatisation and formation of (613), whilst hydrogenation of (611) gave 1,3,4,5,6-penta-*O*-benzyl-*scyllo*-inositol.[570] The oxidation of inositols to inososes by bromine has been studied and the products were characterised as phenylhydrazones or phenylosazones.[571]

Diels–Alder addition of *trans,trans*-1,4-diacetoxybuta-1,3-diene with allyl acetate gave (Scheme 56) the cyclohexene derivative (614), the double

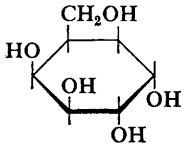

(614)

Scheme 56

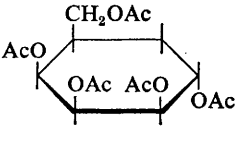
bond of which was unexpectedly inert to addition reactions. It was tentatively suggested that this effect arose from deactivation by π-interaction with the carbonyl group of the acetoxymethyl side chain. However, hydroxylation could be effected with t-butyl hydroperoxide and osmium tetroxide and led eventually to the isolation of the *β-gulo*-'pseudo-hexose'

[570] W. Meyer zu Reckendorf, *Chem. Ber.*, 1968, **101**, 3652.
[571] A. J. Fatiadi, *Carbohydrate Res.*, 1968, **8**, 135.

as its penta-acetate.[572] The α-*galacto*-'pseudo-hexose' (615) was isolated after isomerising the α-*talo* per-acetate (616) with 95% acetic acid, containing sulphuric acid.[573]

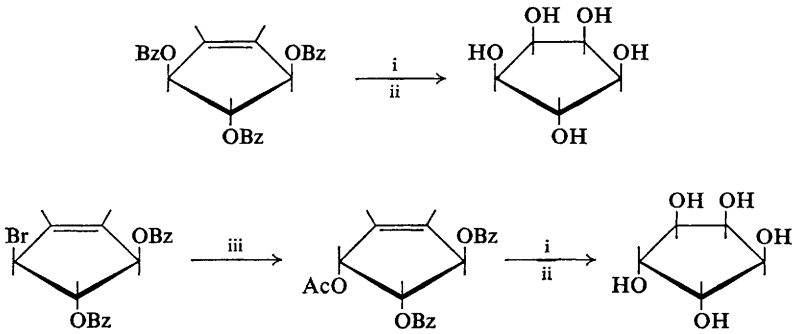


(615)



(616)

Two new cyclopentane-pentols have been prepared as shown in Scheme 57.[574] The synthesis of a cyclityl α-D-mannoside has already been described.[73]



Reagents: i, OsO_4; ii, OH^-; iii, $Et_4N^+OAc^-$

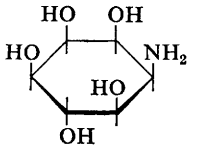
Scheme 57

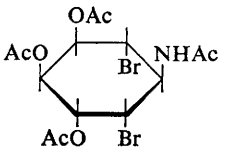
The mass spectra of many cyclohexane polyols have been measured (see p. 200). A detailed study has been made of the n.m.r. signals from *O*- and *N*-acetyl groups of cyclitol derivatives (see p. 193).

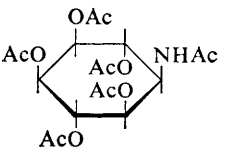
Nitrogen-containing Derivatives

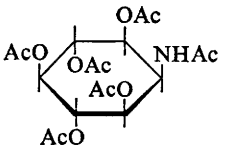
Bromination of *epi*-inosamine-2 (617) with acetyl bromide gave most probably, 1,5-dibromo-1,5-dideoxy-*rac*-inosamine-6, as its per-acetyl derivative (618). N.m.r. studies on the debrominated product did not conclusively establish its configuration and so the reaction of (618) with nucleophiles was studied. When heated with sodium acetate in 2-methoxyethanol it gave (619) and (620) and the hexa-acetyl derivative of (617), whilst reaction with sodium azide and then hydrogenation gave hexa-acetyl-*muco*-inosadiamine-2,3 (621) and hexa-acetyl-*myo*-inosadiamine-2,4 (622), the configurations of which were established by n.m.r. The formation

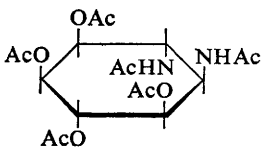
[572] G. E. McCasland, S. Furuta, and L. J. Durham, *J. Org. Chem.*, 1968, **33**, 2835.
[573] G. E. McCasland, S. Furuta, and L. J. Durham, *J. Org. Chem.*, 1968, **33**, 2841.
[574] T. Posternak and G. Wolczunowicz, *Naturwiss.*, 1968, **55**, 82.

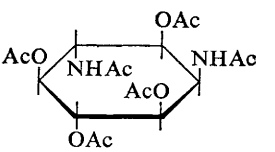

(617)


(618)


(619)

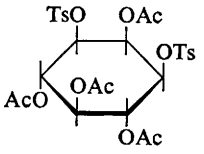

(620)

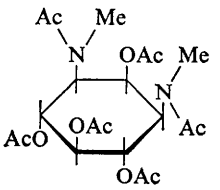

(621)

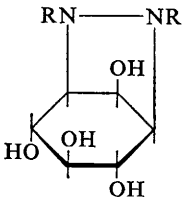

(622)

of the various products was rationalised by considering routes involving oxazolinium and acetoxonium ions as intermediates.[575]

Hydrazinolysis of 2,4,5,6-tetra-*O*-acetyl-1,3-di-*O*-toluene-*p*-sulphonyl-*myo*-inositol (623) followed by catalytic hydrogenation, methylation, and


(623)


(624)

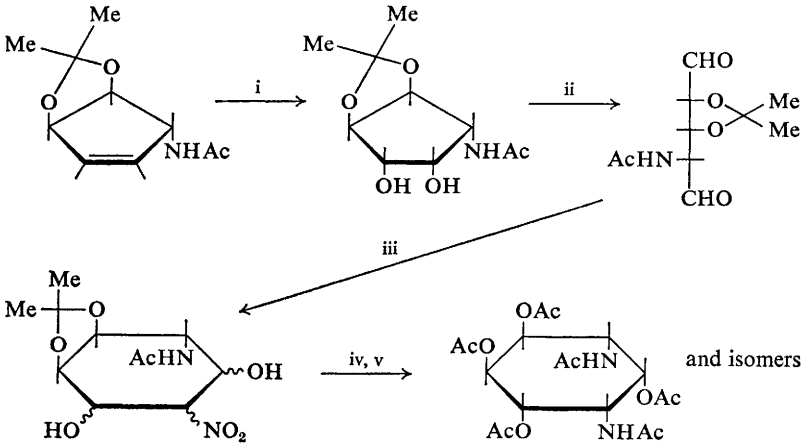

(625) R = H
(626) R = Me

acetylation gave actinamine as its hexa-acetyl derivative (624).[576] In the course of this work the intermediate (625) was isolated and on hydrogenation gave the corresponding diamino-compound. Displacements in the original reaction occurred with participation of the neighbouring *trans*-acetoxy-groups. In a similar reaction, *N,N'*-dimethylhydrazine on 1,3-di-*O*-toluene-*p*-sulphonyl-*myo*-inositol, followed by hydrogenation and acetylation, also gave (624). It was assumed in this case that (626) was the intermediate product.[577]

The full details[578] of the synthesis of 2-deoxystreptamine from *myo*-inosadiamine-1,3, by bromination then reductive de-bromination have been

[575] T. Suami, S. Ogawa, Y. Nakashima, and H. Sano, *Bull. Chem. Soc. Japan*, 1967, **40**, 2958.
[576] T. Suami, S. Ogawa, S. Naito, and H. Sano, *J. Org. Chem.*, 1968, **33**, 2831.
[577] T. Suami and H. Sano, *Tetrahedron Letters*, 1968, 2655.
[578] T. Suami, F. W. Lichtenthaler, S. Ogawa, Y. Nakashima, and H. Sano, *Bull. Chem. Soc. Japan*, 1968, **41**, 1014.

given (Vol. 1, 161). A new synthesis of inosadiamines is based on nitromethane cyclisation of 4-amino-2,3-dihydroxypentodialdo-1,5-oses, that were obtained as shown for the example in Scheme 58.[579] A range of isomers

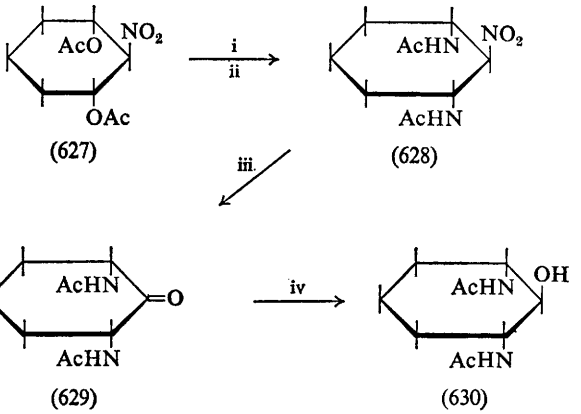


Reagents: i, $KMnO_4$; ii, IO_4^-; iii, $MeNO_2$, EtO^-; iv, H_2–Ni; v, Ac_2O–py

Scheme 58

were isolated and were characterised mainly by determination of the orientation of the acetoxy-groups by n.m.r. chemical shift correlations. The validity of this procedure was critically assessed.

Treatment of the diacetoxydeoxynitrocyclohexane derivative (627) as shown in Scheme 59 allowed the synthesis of (628), *via* unsaturated intermediates, and further reaction as shown gave the ketone (629) and the

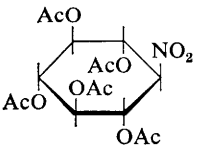


Reagents: i, NH_3; ii, Ac_2O–py; iii, $KMnO_4$; iv, $NaBH_4$

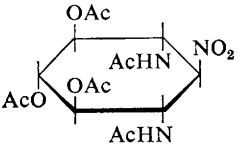
Scheme 59

[579] A. Hasegawa and H. Z. Sable, *J. Org. Chem.*, 1968, **33**, 1604.

alcohol (630). This sequence represents a model for the synthesis of all *trans*-2,6-diaminocyclohexan-1,3,4,5-tetrol(streptamine). As a first step in such a synthesis, (631) was converted into (632) in 76% yield. A similar



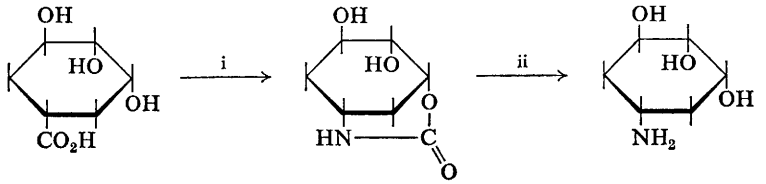
(631)



(632)

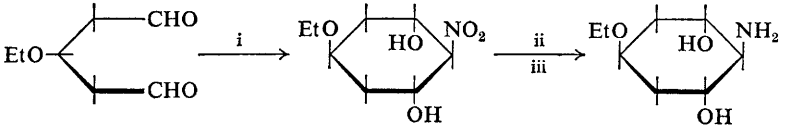
reaction on tetra-*O*-acetyl-1,4-dideoxy-1,4-dinitro-*neo*-inositol gave 2,5-dinitroaniline.[580]

Routes to two isomeric trihydroxycyclohexylamines have been developed, one starting from dihydroshikimic acid (Scheme 60) and the other based on a nitromethane cyclisation (Scheme 61).[581]



Reagents: i, Curtius reaction; ii, NaOH

Scheme 60



Reagents: i, $MeNO_2$–EtO^-; ii, Pt–H_2; iii, Ac_2O–py

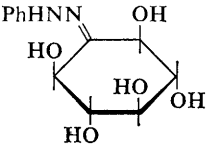
Scheme 61

Catalytic hydrogenation of 4,6-dinitropyrogallol (*cf.* Vol. **1**, p. 161) gave 2-deoxy-*cis*-1,3-inosadiamine as the main product. After *N*-acetylation, the C-4-epimer was obtained by platinum-catalysed oxidation followed by reduction, then by a similar sequence for epimerisation at C-6 to give 2-deoxystreptamine.[582]

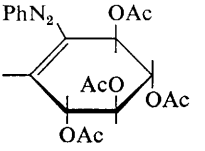
Acetylation of inosose phenylhydrazones has been shown to follow that previously described for aldose phenylhydrazones, in that unsaturated

[580] H. H. Baer and M. C. T. Wang, *Canad. J. Chem.*, 1968, **46**, 2793.
[581] F. W. Lichtenthaler and H. K. Yahya, *Chem. Ber.*, 1968, **101**, 908.
[582] D. Dijkstra, *Rec. Trav. chim.*, 1968, **87**, 161.

phenylazo-derivatives were formed; *e.g.* (633) gave (634).[583] The latter



(633)



(634)

compound had previously been obtained from the reaction of penta-*O*-acetyl-*myo*-inosose-2 with phenylhydrazine in acetic anhydride and the product assigned a phenylhydrazone structure.

[583] A. J. Fatiadi, *Carbohydrate Res.*, 1968, **7**, 89.

20
Antibiotics

Again there has been much activity in this area, and many novel carbohydrate derivatives have been isolated. A text in two volumes covering the mechanism of action and the biosynthesis of antibiotics has appeared; much information relevant to the chemistry of carbohydrate-containing antibiotics is discussed.[584] The proceedings of the seventh International Conference on Antimicrobial Agents and Chemotherapy have been published.[585] A report on aspects of some carbohydrate-containing antibiotics, including lincomycin and daunomycin, has been published, together with an outline of the biosynthesis of streptomycin.[586] The structural studies on kasugamycin have been reviewed.[587]

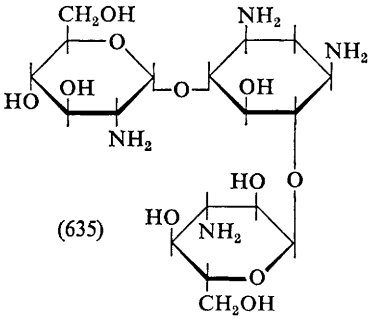
The first example of a naturally occurring nitro-sugar, evernitrose (527), has been isolated after hydrolysis of everninomicins B and D.[490] The sugar component of the macrolide antibiotic azalomycin B has been shown to be 2-deoxy-L-fucose(2,6-dideoxy-L-*lyxo*-hexose).[588] Nogalose, a component of the anthracyclinone antibiotic, nogalamycin, has been shown to be a branched-chain derivative of partial structure (529) by n.m.r. studies. Another component of this antibiotic, $C_8H_{15}NO_3$, was believed to be an amino-sugar.[491] The antibiotic desertomycin appears to be the first with D-mannose as the sole carbohydrate component.[589]

An amino-sugar, of as yet unknown structure, has been isolated from the acid hydrolysate of ristomycin A, ristocetin, actinoidin, and vancomycin.[589a] Compounds with antibiotic activity have been isolated from *Tulipa gesnerania* and shown to be glucosyl esters of structure (127) and (128), named 1-tuliposide A and B, respectively. Also isolated were inactive compounds, shown to be the 6-*O*-isomers of (127) and (128).[226]

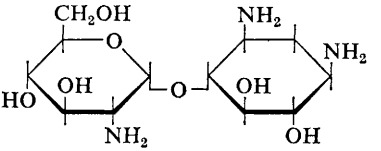
[584] 'Antibiotics,' ed., D. Gottlieb and P. D. Shaw, Springer-Verlag, Berlin, 1967.
[585] 'Antimicrobial Agents and Chemotherapy, 1967,' ed. G. L. Hobby, Amer. Soc. for Microbiology, 1968, 770pp.
[586] S. A. Goulden, *Manuf. Chemist*, 1968, **39**, 70.
[587] Y. Suhara, K. Maeda, H. Umezawa, and M. Ohno, *Adv. Chem. Ser. No.* 74, 1968, 15.
[588] S. Takahashi, M. Kurabayashi, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1657.
[589] R. Bognár, F. Sztaricskai, L. Somogyi, M. Puskás, and S. Makleit, *Acta Chim. Acad. Sci. Hung.*, 1968, **56**, 53.
[589a] N. N. Lomakina, I. A. Spiridonova, R. Bognár, M. Puskás, and F. Sztaricskai, *Antibiotiki*, 1968, **13**, 975.

The amino-sugar component of pimaricin, mycosamine, has been shown to be present in the pyranose form.[590]

Much work continues to be done on kanamycin by various groups of Japanese workers. An *X*-ray diffraction study on its sulphate has confirmed the chemically assigned structure.[591] Kanamycin C (635) has been synthesised by coupling 3-amino-3-deoxy-D-glucose with paromamine (636),



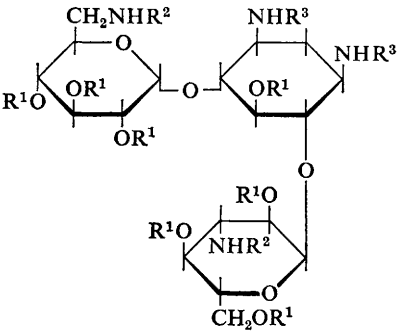
(635)



(636)

using suitably blocked derivatives.[592, 593] Since (636) has been prepared previously, this constitutes a total synthesis of (635).

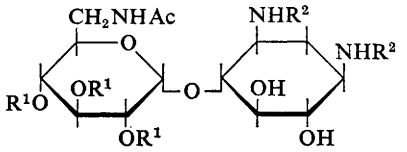
A preliminary report on the total synthesis of kanamycin A (637) has appeared. The isopropylidene derivative of *N,N'*-dicarbobenzoxy-2-deoxystreptamine was condensed with 6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-D-glucopyranosyl chloride in a modified Koenigs–Knorr synthesis



	R^1	R^2	R^3
(637)	H	H	H
(638)	CH_2Ph	Ac	CO_2CH_2Ph

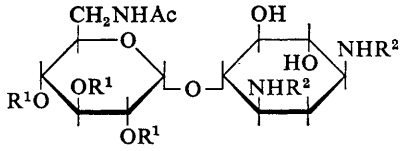
[590] W. E. Meyer, *Chem. Comm.*, 1968, 470.
[591] G. Koyama, Y. Iitaka, K. Maeda, and H. Umezawa, *Tetrahedron Letters*, 1968, 1875.
[592] S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 162.
[593] S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, *Bull. Chem. Soc. Japan*, 1968, **41**, 533.

to give compounds (639) and (640). Condensation of (639) with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-D-glucopyranosyl chloride as before



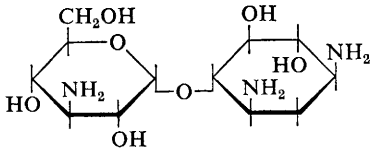
(639)

$R^1 = CH_2Ph$
$R^2 = CO_2CH_2Ph$



(640)

gave (638), which could be converted into (637), identical in all respects with the natural material.[594] A paper also appeared on the synthesis of the pseudo-disaccharide (641), a component of kanamycin A,[595] which was

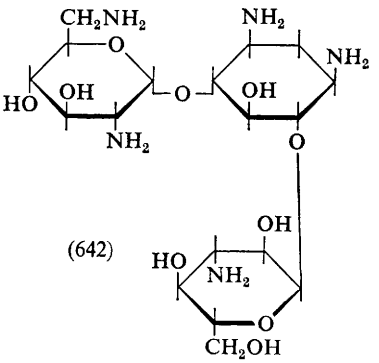


(641)

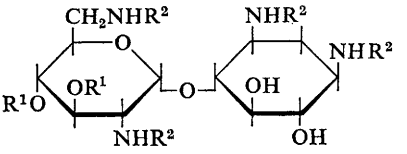
then subsequently coupled after blocking groups had been added, with 6-acetamido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride, to give, after deblocking, kanamycin A.[596] The total synthesis of kanamycin B (642) has been achieved by condensation of (643) with (644).[597]

Work has continued on the chemical modification of kanamycin; sixteen tetra-*N*-phenylalkyl derivatives have been described.[598] Partial toluene-*p*-sulphonylation of tetra-*N*-acetylkanamycin gave the di-*O*-sulphonyl derivative (644a) as the main product. Treatment of this crude product with sodium azide in DMF gave the 'manno-kanamycin' derivative (645), after chromatography. Reduction gave the amino-compound which could be converted to (647). Reaction of the crude (644a) with hydrazine gave, again after chromatography, (646), which on reduction gave (647).[599]

[594] M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno, and D. Nishimura, *Tetrahedron Letters*, 1968, 623.
[595] S. Umezawa, K. Tatsuta, E. Kitazawa, and S. Koto, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 365.
[596] S. Umezawa, K. Tatsuta, and S. Koto, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 367.
[597] S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 424.
[598] A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 340.
[599] S. Inouye, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 1888.

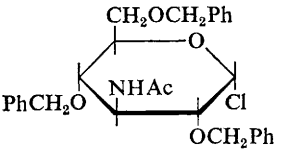


(642)

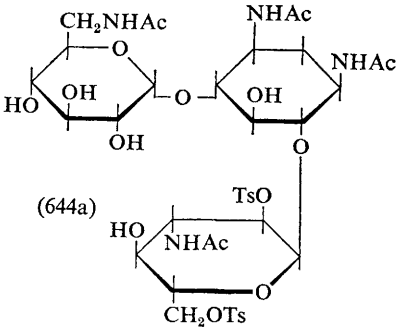


$R^1 = CH_2Ph$, $R^2 = CO_2CH_2Ph$

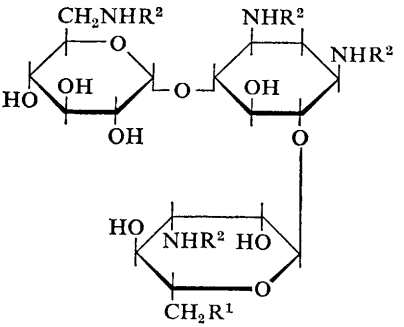
(643)



(644)

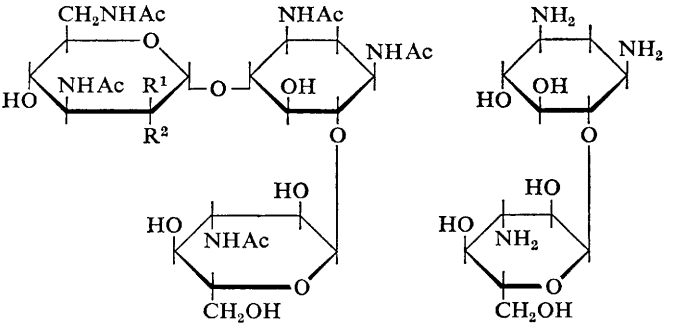


(644a)



(645) $R^1 = N_3$, $R^2 = Ac$
(646) $R^1 = NHNH_2$, $R^2 = H$
(647) $R^1 = NH_2$, $R^2 = H$

A novel application of the nitromethane cyclisation reaction has been applied to the product from periodate oxidation of tetra-*N*-acetylkanamycin. The resulting nitro-derivative was subsequently reduced, and deacetylated with hydrazine to give '3′-amino-3′-deoxykanamycin' (648) and '3′-amino-3′-deoxymanno-kanamycin' (649).[600] Direct treatment of the 3′-deoxy-3′-nitro-derivative with hydrazine gave (650). Further studies have been



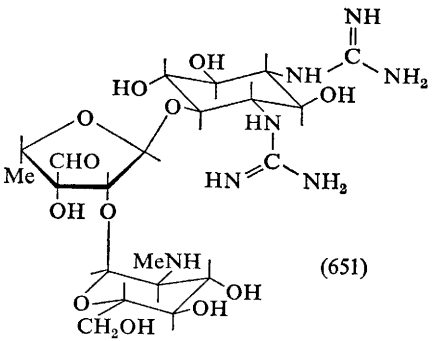
(648) $R^1 = H$, $R^2 = OH$
(649) $R^2 = OH$, $R^2 = H$

(650)

made on the deactivation of kanamycin and paromamine by an enzymic extract of *E. coli* (*cf.* Vol. 1, p. 165).[601]

Deactivation of paromamine by *Pseudomonas aeruginosa* has been shown to result from phosphorylation at the 3′-position and the product was isolated and characterised.[602]

An *X*-ray structural study on streptomycin oxime selenate has shown the parent compound to have the structure and absolute configuration shown

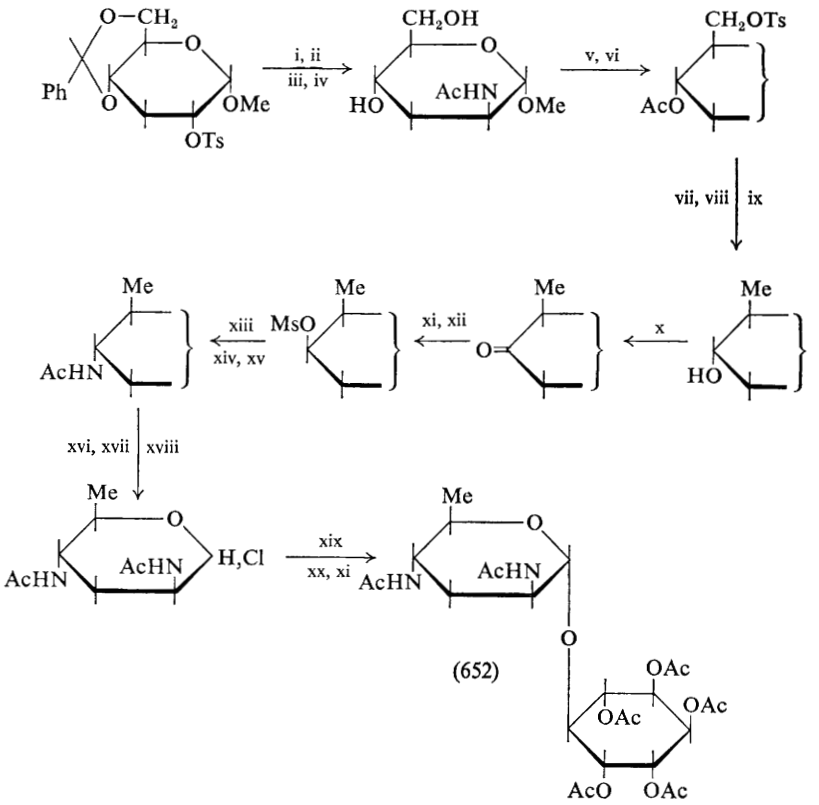


(651)

[600] S. Inouye, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 573.
[601] S. Kondo, M. Okanishi, R. Utahara, K. Maeda, and H. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 22.
[602] K. Maeda, S. Kondo, M. Okanishi, R. Utahara, and H. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1968, **21**, 458.

in (651), which definitely resolved the nature of the glycosidic linkages.[603] Streptomycin inactivated by an enzyme preparation from *E. coli* in the presence of adenosine triphosphate and magnesium ions was found to have had the hydroxy-group at C-3 of the *N*-methyl-L-glucosamine moiety substituted by an adenylic acid group (phosphodiester linkage).[604]

The synthesis of kasugabiosamine (652) as its hepta-acetate has been achieved as shown in Scheme 62, starting from the known methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside.[605] A



Reagents: i, NaN_3–DMF; ii, H_2–Pt; iii, acetylation; iv, aq. H_2SO_4; v, TsCl–py; vi, Ac_2O–py; vii, NaI–Me_2CO; viii, H_2–Ni; ix, MeO^-; x, $H_2Cr_2O_7$; xi, H_2–Pt; xii, MsCl–py; xiii, NaN_3–DMF; xiv, H_2–Pt; xv, acetylation; xvi, 5N-HCO_2H; xvii, Ac_2O–py; xviii, HCl–AcOH; xix, 1,2:3,4-di-*O*-isopropylidene-(+)-inositol; xx, aq. AcOH; xxi, Ac_2O–py

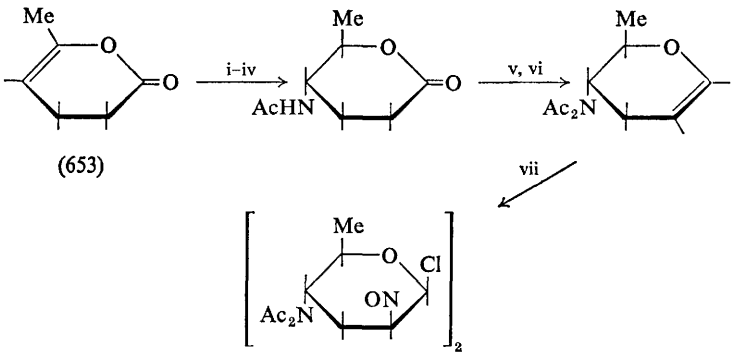
Scheme 62

[603] S. Neidle, D. Rogers, and M. B. Hursthouse, *Tetrahedron Letters*, 1968, 4725.

[604] S. Takasawa, R. Utahara, M. Okanishi, K. Maeda, and H. Umezawa, *J. Antibiotics (Japan)*, *Ser. A*, 1968, **21**, 477.

[605] M. Nakajima, H. Shibata, K. Kitahara, S. Takahashi, and A. Hasegawa, *Tetrahedron Letters*, 1968, 2271.

second independent synthesis of (652) used the synthesis shown in Scheme 63 for the diamino-sugar component, starting from the dihydropyran (653),



(653) (654)

Reagents: i, NOCl; ii, H_2O; iii, H_2–Pt; iv, Ac_2O; v, LAH; vi, Ac_2O–py; vii, NOCl

Scheme 63

by way of the nitroso-glycosyl chloride (654); this, with alcohols in the presence of silver or mercury salts, gave α-glycosides which, on reduction with hydrogen over platinum, gave the 2-amino-2-deoxy-products. Use of 1,2:3,4-di-*O*-isopropylidene-D-inositol afforded a means of obtaining (652). It is of interest that the condensation of the asymmetric inositol derivative with the racemic glycosyl chloride (654) provided a means of resolving the latter, and a fully resolved product identical with the natural product was obtained.[606]

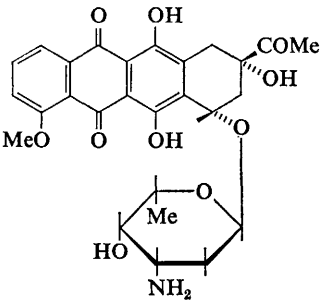
Pyrolitic elimination of *N,N*-dimethylhydroxylamine from the amine oxides of the macrolide antibiotics erythromycin A and B led to the corresponding unsaturated compounds with a C-3, C-4 double bond in the moiety derived from the amino-sugar; hydrogenation gave the de(dimethylamino)-derivatives of the original antibiotics. Methanolysis of the unsaturated compounds gave the unsaturated glycosides (655), which with the corresponding dihydro-derivatives (656) were characterised by n.m.r.[607]

(655) (656)

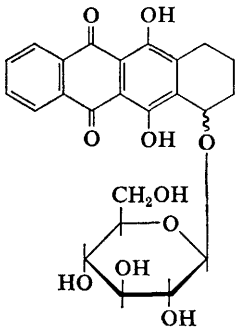
[606] Y. Suhara, F. Sasaki, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.*, 1968, **90**, 6559.

[607] P. H. Jones and E. K. Rowley, *J. Org. Chem.*, 1968, **33**, 665.

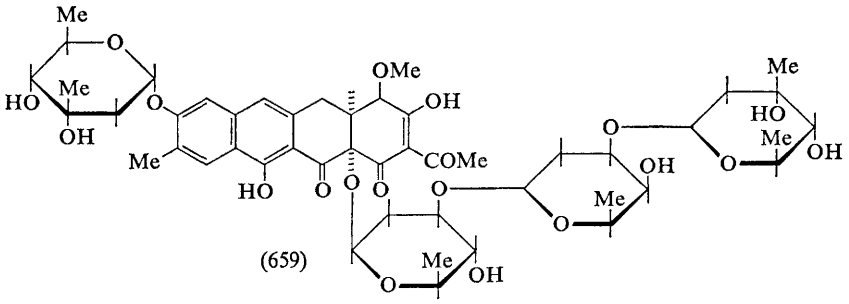
Several papers have appeared on the leucomycins,[608-610] and on daunomycin, which has been shown to have structure (657) by two different



(657)



(658)



(659)

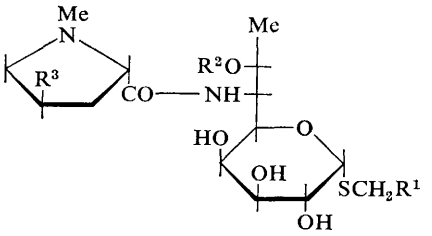
groups.[611-613] The synthesis of the diastereoisomeric daunomycin analogues (658) in which D-glucose was the sugar moiety has been reported.[614] A new antibiotic, chromocyclomycin has been shown to contain the sugars, mycarose, olivose (synonymous with chromose C, canarose), and oliose (deacetyl chromose D) in a 2 : 1 : 1 ratio, and to have the structure (659).[615]

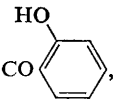
[608] S. Omura, M. Katagiri, and T. Hata, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 199, 272.
[609] S. Omura, M. Katagiri, H. Ogura, and T. Hata, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1167, 1181.
[610] S. Omura, M. Katagiri, I. Umezawa, K. Komiyama, T. Maekawa, K. Sekikawa, A. Matsumae, and T. Hata, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 532.
[611] F. Arcamone, G. Franceschi, P. Orezzi, S. Penco, and R. Mondelli, *Tetrahedron Letters*, 1968, 3349.
[612] F. Arcamone, G. Cassinelli, G. Franceschi, and R. Mondelli, *Tetrahedron Letters*, 1968, 3353.
[613] R. H. Iwamoto, P. Lim, and N. S. Bhacca, *Tetrahedron Letters*, 1968, 3891.
[614] J. P. Marsh jun., R. H. Iwamoto, and L. Goodman, *Chem. Comm.*, 1968, 589.
[615] Yu. A. Berlin, M. N. Kolosov, I. V. Vasina, and I. V. Yartseva, *Chem. Comm.*, 1968, 762.

Work on avilamycin,[615a] and curamycin[615b] has shown them to be similar and to contain as sugar components, 2-deoxy-D-rhamnose, 2,6-di-*O*-methyl-D-mannose (curamicose), 4-*O*-methyl-D-fucose (curacose), and L-lyxose.

Details have been given elsewhere of the synthesis of the antibiotic components 6-deoxy-D-*arabino*-hexofurano-5-ulose (hygromycin A),[518] arcanose (lankomycin),[493] and perosamine (perimycin),[281, 321] as well as of nojirimycin.[440, 441]

Largely by comparison of degradation products with those from lincomycin, the related antibiotics celesticetin and desalicetin have been shown to have structures (660) and (661). A derivative possessing the lincomycin





(660) $R^1 = CH_2OCO$ ⟨benzene⟩, R^2 = Me, R^3 = H

(661) $R^1 = CH_2OH$, R^2 = Me, R^3 = H

feature of having R^3 = *n*-propyl was synthesised to combine features of the latter antibiotic and celesticetin and it showed antibacterial activity between that of the two antibiotics.[616] An investigation has been carried out on the acid-catalysed hydrolysis of *p*-substituted 3,4-*O*-benzylidene-acetals of lincomycin in the hope of finding readily-cleaved derivatives. Kinetic studies showed that the hydrolyses were first order and indicated that they conformed with the generally accepted mechanism for acetal hydrolysis. As expected, electron-releasing substituents facilitated the removal of the group.[617] Methods for the analysis of lincomycin based on g.l.c. of TMS derivatives have been developed.[618, 619]

[615a] F. Buzetti, F. Eisenberg, H. N. Grant, W. Keller-Schierlein, W. Voser, and H. Zaehner, *Experientia*, 1968, **24**, 320.

[615b] E. G. Gros, V. Deulofeu, O. L. Galmarini, and B. Frydman, *Experimentia*, 1968, **24**, 323.

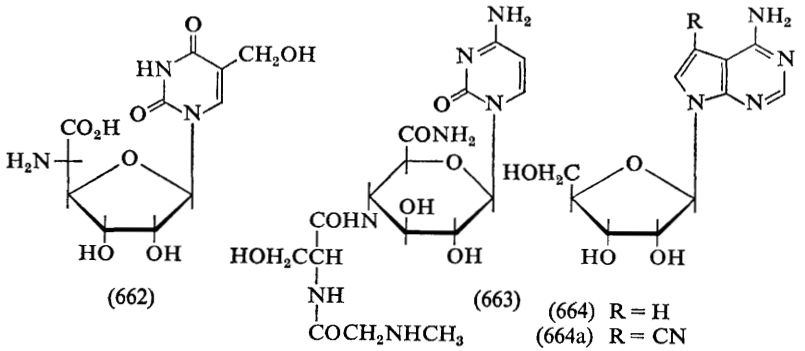
[616] H. Hoeksema, *J. Amer. Chem. Soc.*, 1968, **90**, 755.

[617] M. J. Taraszka and W. Morozowich, *J. Org. Chem.*, 1968, **33**, 2349.

[618] M. Margosis, *J. Chromatog.*, 1968, **37**, 46.

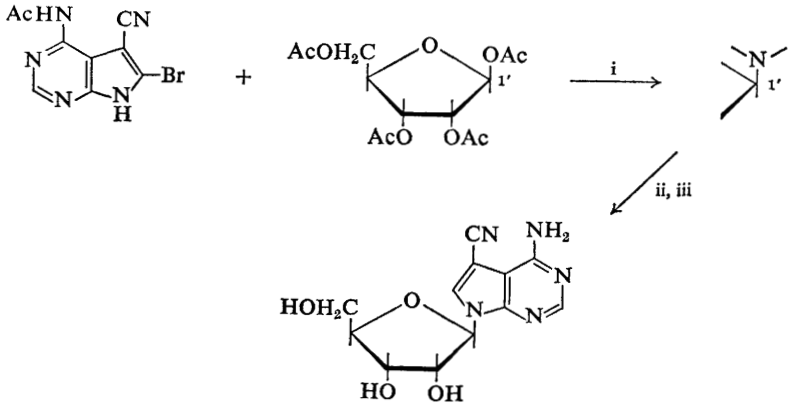
[619] R. L. Houtman, D. G. Kaiser, and A. J. Taraszka, *J. Pharm. Sci.*, 1968, **57**, 693.

Chemical and *X*-ray studies have shown that polyoxin C has the 5′-amino-5′-deoxyallofuranosyl structure (662).[620] By a detailed examination of the amino-sugar moiety, the nucleoside antibiotic gougerotin has been shown to be (663)[621] (*cf.* ref. 318).



(662) (663) (664) R = H (664a) R = CN

Difficulties encountered in the isolation of the aglycone from the 7-deaza-adenine nucleoside antibiotics tubercidin (664) and toyocamycin (664a) have been overcome by carrying out Barry degradations (periodate oxidation followed by reaction of the product with phenylhydrazine). Positions C-1 and C-2 of the ribosyl moiety were isolable as glyoxal bisphenylhydrazone.[622] The latter nucleoside (664a) has been synthesised as shown in Scheme 64[623] and further it was shown to be identical with unamycin

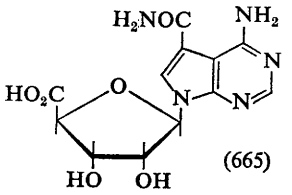


Reagents: i, 175°, $(p\text{-}NO_2C_6H_4O)_2P(O)OH$; ii, MeOH–$NH_3$; iii, H_2–Pd

Scheme 64

[620] K. Isono and S. Suzuki, *Tetrahedron Letters*, 1968, 203; K. Asahi, T. Sakurai, K. Isono, and S. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1046.
[621] J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Letters*, 1968, 6029.
[622] T. Uematsu and R. J. Suhadolnik, *J. Org. Chem.*, 1968, **33**, 726.
[623] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1968, **90**, 524.

B, 'antibiotic E-212', and vengicide. A new nucleoside antibiotic sangivamycin has been identified as closely related to the above nucleosides and to have structure (665).[623, 624]



(665)

The β-D-ribo-furanoside and -pyranoside of 4-amino-5-cyano-6-methylmercaptopyrrol[2,3-*d*]-pyrimidine, both analogues of toyocamycin, have been synthesised by the fusion method.[625]

The nucleoside antibiotic nebularine and its isomer isonebularine have been synthesised *via* a fusion reaction of purine and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose. D-Xylofuranosyl, D-ribopyranosyl, and D-glucopyranosyl analogues of the antibiotic were similarly prepared.[626] Angustmycin A (decoyinine) has been synthesised from psicofuranine as shown in Scheme 37.[482] Several derivatives of formycin have been synthesised and examined for biological activity.[627]

The problems involved in the synthesis of α-glycosidic linkages, necessary for the total synthesis of some antibiotics, have received consideration.[73, 86, 628] A paper has also appeared on the introduction of amino-groups after the formation of the glycosidic linkage.[629]

Sixty-seven semi-synthetic coumermycins (noviose-based compounds) have been described; all had antibiotic properties.[630, 631]

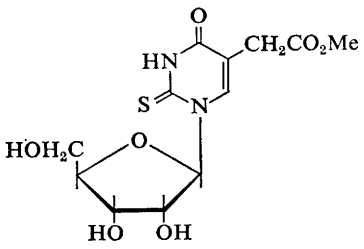
[624] K. V. Rao, *J. Medicin. Chem.*, 1968, **11**, 939.
[625] H. Iwamura and T. Hashizume, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1010
[626] H. Iwamura and T. Hashizume, *J. Org. Chem.*, 1968, **33**, 1796.
[627] T. Kunimoto, T. Wakashiro, I. Okamura, T. Asajima, and M. Hori, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 468.
[628] H. Shibata, I. Takeshita, N. Kurihara, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1006.
[629] M. J. Mohlenkamp and L. Anderson, *J. Org. Chem.*, 1968, **33**, 3163.
[630] J. G. Keil, I. R. Hooper, M. J. Cron, O. B. Fardig, D. E. Nettleton, F. A. O'Herron, E. A. Ragan, M. A. Rousche, H. Schmitz, R. H. Schreiber, and J. C. Godfrey, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 551.
[631] H. Schmitz, R. L. DeVault, C. D. McDonnell, and J. C. Godfrey, *J. Antibiotics (Japan), Ser. A*, 1968, **21**, 603.

21
Nucleosides

Recent work on nucleosides and nucleotides has been reviewed.[632]

Naturally Occurring Nucleosides

Papers on nucleoside antibiotics are described in Chapter 20 (p. 179). A nucleoside isolated from yeast RNA has been tentatively assigned structure (666),[633] and 5′-*S*-methyl-5′-thioadenosine has been isolated from *E. coli*.[634]



(666)

Synthesis

Many papers have appeared on this topic and those compounds which were prepared by standard methods will in general not be listed here. Many papers, principally from the groups of Ulbricht and of Wagner, have described work on $O \rightarrow N$-glycosyl rearrangements of nucleosides; these papers may be readily located through *Chemical Abstracts*.

The Hilbert–Johnson procedure for nucleoside synthesis by condensation of 2,4-dialkoxypyrimidines with acylhalogenoses has been reviewed.[635]

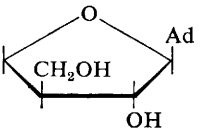
The fusion synthesis continues to be developed and has been applied to the preparation of 2′-deoxy-[636, 637] and 3′-deoxy[637]-derivatives, and also

[632] B. Shimizu, *Ann. Sankyo Res. Lab.*, 1967, **19**, 1 (*Chem. Abs.*, 1968, **68**, 96054y).
[633] L. Baczynskyj and K. Biemann, *Science*, 1968, **159**, 1481.
[634] T. Ming Chu, M. F. Mallette, and R. O. Mumma, *Biochemistry*, 1968, **7**, 1399.
[635] J. Pliml and M. Prystas, *Adv. Heterocyclic Chem.*, 1967, **8**, 115.
[636] J. A. Montgomery and K. Hewson, *J. Medicin. Chem.*, 1968, **11**, 48.
[637] K. Antonakis and F. Leclercq, *Compt. rend.*, 1968, **267***C*, 1343.

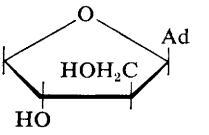
used as a key step in a new synthesis of 7-(β-D-ribofuranosyl)-purines, prepared *via* imidazole derivatives.[638] Fusion of 1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose with theophylline gave the unsaturated derivative (499), rather than the expected rhamnosyl nucleoside.[477] The fusion synthesis using per-acetylated glycals, to give 2,3-unsaturated nucleosides, has been used in the preparation of a guanosine analogue of blasticidin S.[465] 3,4-Di-*O*-acetyl-D-arabinal and 6-chloropurine gave 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribofuranosyl) purine and its β-anomer.[466]

Several papers have appeared on the synthesis of 3′-deoxynucleosides,[460, 461, 463] including the 7-isomer (466) of cordycepin.[462]

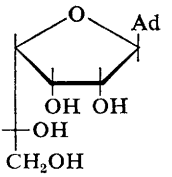
The syntheses of the branched-chain nucleosides, 2′- and 3′-*O*-methyladenosine have been reported.[497, 498] The same group have also prepared the 5′-*C*-methyl-6′-deoxynucleoside (537).[499] Full details (*cf.* Vol. **1**, p. 135) have been given of the preparation of the branched-chain nucleosides (667)



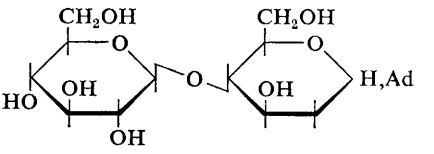
(667)



(668)



(669)



(670)

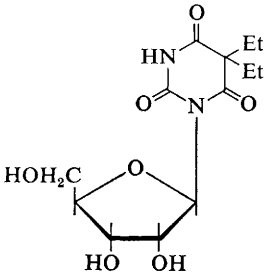
and (668).[508] Another example of this class has been prepared, namely (539).[500]

The previously developed procedure for using aldono-γ-lactones in the synthesis of furanoid derivatives has been applied to the preparation of 9-(β-D-gulofuranosyl)adenine (669).[542] Several new classes of nucleosides have been described, including derivatives of D-*glycero*-D-*gulo*-pyranose,[638a] a 2′-deoxy-disaccharide nucleoside (670),[639] and 1-ribofuranosyl hypoxanthine.[640] Further examples of 2-amino-2-deoxyglycosyl nucleosides have been described,[341] in the synthesis of which the acetyl group was used for protecting the amino-function.

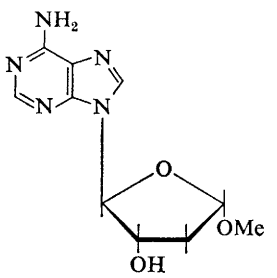
[638] R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1968, **90**, 2661.
[638a] L. M. Lerner, *J. Pharm. Sci.*, 1968, **57**, 1263.
[639] L. M. Lerner, *J. Medicin. Chem.*, 1968, **11**, 912.
[640] J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, 1968, **5**, 741.

Condensation of N^6-octanoyl-adenine with 1,2,3,5-tetra-*O*-acetyl- or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in ethylene dichloride, or chlorobenzene, in the presence of Friedel–Craft catalysts, followed by hydrolysis of acyl groups gave adenosine; no α-anomer was found. Pyranosyl isomers were similarly obtained; some experiments were also carried out with N^6-palmitoyl-adenine.[641] The reaction was believed to occur *via* a 1,2-acyloxonium ion which was specifically attacked to give the β-product.

The derivatives with sulphur in the sugar ring (423) and (424) have been described.[436] The synthesis of 1-(β-D-ribofuranosyl)-5,5-diethylbarbituric acid (671) and of its 5-ethyl-5-phenyl analogue has been achieved by reaction



(671)

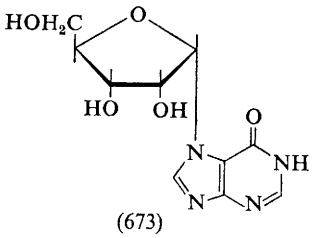


(672)

of the appropriate malonic acid chloride with 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-urea, and removal of the blocking groups.[642] The preparation of the 'reverse nucleoside' (672) has been described.[476] Nucleoside derivatives of 2-deoxy-D-*arabino*-hexofuranose[643] and of 2-deoxy-D-*ribo*-hexofuranose[459] have been reported.

Several papers have appeared on α-nucleosides. Methyl β-D-ribofuranoside, as its boron trichloride complex, and N^6-acyl-purines in the presence of pyridine gave a good yield of the α-nucleoside; the method was used in the synthesis of α-adenosine and α-guanosine.[644] 2,3,5-Tri-*O*-benzyl-D-ribofuranosyl chloride has been found to be a good reagent for the synthesis of α-nucleosides; for example, condensation with 5,6-dimethylbenzimidazole gave the α-product in 66% yield. Use of the analogous benzoyl derivative gave 51% of the β-product, but also 28% of the α-anomer, showing that the '*trans*' rule does not apply exclusively and can thus be unreliable as a means of assigning anomeric configuration to synthetic nucleosides.[645] The nucleoside (673) occurring in Factor G has been synthesised, with its

[641] Y. Furukawa and M. Honjo, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1076.
[642] M. Sprinzl, J. Farkaš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 4280.
[643] W. E. Dick jun., B. G. Baker and J. E. Hodge, *Carbohydrate Res.*, 1968, **6**, 52.
[644] Y. Furukawa, K. Imai, and M. Honjo, *Tetrahedron Letters*, 1968, 4655.
[645] J. D. Stevens, R. K. Ness, and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 1806.



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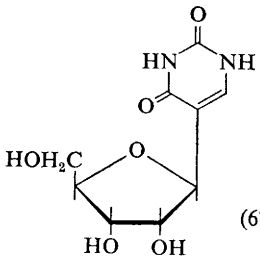


(674)

β-anomer, by treatment of 9-propenyl-hypoxanthine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in DMF; the anomers were formed in similar proportions.[646] Condensation of the silver salt of hypoxanthine with acetobromoglucose in refluxing xylene led to the isolation of the 7α-, 7β-, 9α-, and 9β-products, the α-anomers being new compounds.[647]

Full details have been published of the synthesis of the *C*-nucleoside, pseudouridine (674), from the condensation of 2,4-di-t-butoxypyrimidin-5-yl lithium with 2,4:3,5-di-*O*-benzylidene-D-ribose, followed by mild acid treatment which removed all of the blocking groups and caused cyclisation. The α- and β-furanosides were isolated in 8 and 18% yield, respectively, together with a trace of pyranosyl isomers.[648] The preparations of both D- and L-forms of the thymine derivative of 2-deoxy-*erythro*-pentopyranose have been described.[649]

Physical Measurements

Several papers have appeared on *X*-ray crystallographic structural studies on nucleosides and their derivatives; they are discussed on p. 200. Reports on c.d. and o.r.d. spectra are given on p. 205. The n.m.r. spectra of nucleosides continue to be investigated in some detail and work has been done on 2′-halogeno-derivatives [313] and 5-fluoropyrimidine nucleosides,[650] α-nucleosides,[651] branched-chain nucleosides,[497, 498] and mono- and di-nucleotides.[652] The mass spectra of TMS derivatives of some nucleosides [653] and the e.s.r. spectra of irradiated 2′-deoxy-adenosine [654] have been studied.

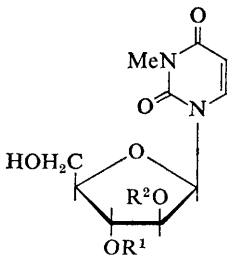
Esters

Papers on nucleoside phosphates have been described in Chapter 6 (p. 57). The synthesis of thymidine 5′-nitrate [296] and papers on the partial esterification of nucleosides [211, 229] have been described in detail elsewhere.

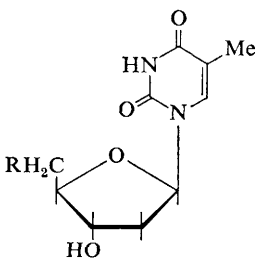
[646] J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, 1968, **5**, 303.
[647] G. T. Rogers and T. L. V. Ulbricht, *Tetrahedron Letters*, 1968, 1025.
[648] D. M. Brown, M. G. Burdon, and R. P. Slatcher, *J. Chem. Soc.* (*C*), 1968, 1051.
[649] G. Etzold, R. Hintsche, and P. Langen, *Chem. Ber.*, 1968, **101**, 226.
[650] R. J. Cushley, I. Wempen, and J. J. Fox, *J. Amer. Chem. Soc.*, 1968, **90**, 709.
[651] K. Onodera, S. Hirano, and F. Masuda, *Carbohydrate Res.*, 1968, **7**, 27.
[652] F. E. Hruska and S. S. Danyluk, *J. Amer. Chem. Soc.*, 1968, **90**, 3266.
[653] J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger, and R. N. Stillwell, *J. Amer. Chem. Soc.*, 1968, **90**, 4182.
[654] J. L. Lichter and W. Gordy, *Proc. Nat. Acad. Sci., U.S.A.*, 1968, **60**, 450.

Other Derivatives

Papers have appeared on the benzylation of nucleosides,[156, 157] including one describing the use of 3,5-di-*O*-benzyl-β-D-ribofuranose for the synthesis of nucleosides having different substituents at C-2′, and C-3′, and C-5′.[310] The partially methylated derivatives (675) and (676) of spongouridine were



(675) R^1 = H, R^2 = Me
(676) R^1 = Me, R^2 = H



R = OH → OTs → I → CN → CH_2NH_2 → CH_2OH
(677) (678)

prepared *via* the 3,5-di-*O*-trityl- and the 2,2′-anhydro-compounds respectively. In the latter case, selective methylation occurred (Kuhn technique) at the less expected secondary site. The positions of the methyl ether groups were determined using hydroxy n.m.r. resonances in [$^2H_6$] DMSO solutions. The n.m.r. spectra also indicated that anomeric configurations of methylated nucleosides can be allocated on the basis of the chemical shifts of the methyl protons.[655]

A preliminary report of the synthesis of the puromycin analogue (345) *via* a nitromethane cyclisation has been described.[400] Chain extension of thymidine to homothymidine has been carried out by the sequence (677) → (678) [656] Full details of the synthesis (see Vol. **1**, p. 72) of 3′-thioadenosine have been published,[657] and the syntheses of 5′-thio- and 5′-amino-5′-deoxy-derivatives of inosine have been reported.[658]

The syntheses of 9-(3-deoxy-3-fluoro-β-D-xylofuranosyl)adenine and its α-*arabino*-isomer have been described,[312] as has that of 5′-phosphoric acid derivatives.[554a] The structures of the photohydration products of cytidine and uridine have been investigated.[391]

The 2,4-dinitrobenzenesulphenyl ester has been used as a base-labile protecting derivative for the 5′-hydroxy-group; it could be removed with hydrogen sulphide in pyridine.[659]

[655] J. F. Codington, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, 1968, **33**, 466.
[656] G. Etzold, G. Kowollik, and P. Langen, *Chem. Comm.*, 1968, 422.
[657] K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, 1968, **33**, 1783.
[658] A. Hampton, M. Bayer, V. S. Gupta, and S. Y. Chu, *J. Medicin. Chem.*, 1968, **11**, 1229.
[659] G. W. Grams and R. L. Letsinger, *J. Org. Chem.*, 1968, **33**, 2589.

22
Oxidation* and Reduction

Periodate Oxidation

A review of the periodate oxidation of glycosylamines has appeared.[374] Warning has been given that concentrated solutions of periodic acid in DMSO can be explosive. It was recommended therefore that oxidation of carbohydrates in this solvent should be performed using dilute solutions of the oxidant, prepared by adding it to DMSO.[660]

The interaction of periodate ion and cyclohexan-1,2,3-triols has been investigated and a specific complex was shown to be formed by the *cis,cis*-isomer, in agreement with previously reported work on pyranose triols. The complex was most stable under basic conditions.[661] The periodate oxidation of aldose dithioacetals and of aldose disulphones has been examined, and shown to be more characteristic of the ring-size of the latter if carried out at 3°.[423] The products from the periodate oxidation of a large number of osazones have been isolated and characterised.[362] Studies on the oxidation of sulphate esters of free sugars showed that the 2-esters, in contrast to others, were oxidised in the open-chain form.[265] Methods for the quantitative analysis of periodate ion have been studied (see p. 211).

DMSO-based Oxidations

Use of such reagents continues to be a favoured method of oxidation (see, for example, refs. 14, 194, 328, 330, 514–517, 519, 521, 539, 554*a*, 569, and 628). Full details have now appeared of Onodera's investigations into the DMSO–phosphorous pentoxide system.[662] The best conditions were found to be with DMF as solvent, 3–4 moles of DMSO and 1·5–2 moles of phosphorous pentoxide at 65–70°. The reagent was shown to be very similar to the DMSO–DCC and DMSO–acetic anhydride systems. Ester, acetal, acetamido, and glycosidic bonds were unaffected by the reagent; *N*-arylglycosylamines, however, were not stable. A wide variety of blocked carbohydrates bearing a free hydroxy-group were cleanly oxidised in good

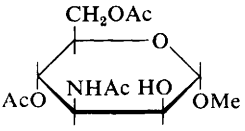
[660] J. J. M. Rowe, K. B. Gibney, M. T. Yang, and G. G. S. Dutton, *J. Amer. Chem. Soc.*, 1968, **90**, 1924.
[661] D. Dijkstra, *Rec. Trav. chim.*, 1968, **87**, 181.
[662] K. Onodera, S. Hirano, and N. Kashimura, *Carbohydrate Res.*, 1968, **6**, 276.

* See also Chapter 16.

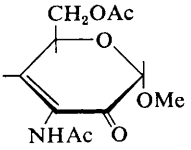
yield. The reagent oxidised 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, which was unaffected by the DMSO–DCC system. It was shown that oxidation of an epimeric pair of hydroxy-groups occurred at the same rate.

Oxidation of primary alcohols to aldehyde groups does not occur with DMSO–acetic anhydride;[663, 664] for example, oxidation of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose gave predominantly the 6-methylthiomethyl ether.[663] However, use of the Pfitzner–Moffatt reagent for this oxidation gave the expected aldehyde;[335, 513] other successful oxidations of primary alcohol to aldehyde have been reported with this latter reagent (for example, refs. 514, 554*a*, and 664).

Attempted Pfitzner–Moffatt oxidation of methyl 3-acetamido-4,6-di-*O*-acetyl-3-deoxy-α-D-mannopyranoside (679) gave the unsaturated keto-sugar



(679)



(680)

(680).[628] A further report of epimerisation accompanying DMSO-based oxidation has been given, this time in an acyclic derivative.[539]

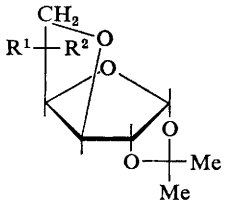
Two papers on the use of DMSO–boron trifluoride have already been mentioned[526, 527] as has a paper describing an unsuccessful attempt to perform an intramolecular oxidation–reduction by use of the substituted DMSO-derivatives (410) and (411).[429]

Platinum-catalysed Oxidation

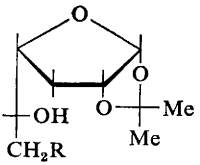
A series of 1,4-anhydro-hexitols have been oxidised with oxygen over a platinum catalyst and the positions of selective oxidation determined. The order of preference was primary hydroxyl ≈ quasi-axial > quasi-equatorial, secondary side-chain hydroxyl. Further oxidation of the cyclic ketonic products caused ring-opening and the formation of dicarboxylic acids.[665] Oxidation of the C-5-epimers (681) and (682) gave the same C-5-ketone, isolated as its *gem*-diol derivative; the *ido*-isomer (682) was oxidised the faster. The corresponding glucurono-3,6-lactone also gave a 5,5-*gem*-diol.[666]

Although the 6-deoxy-derivative (683) was oxidised by DMSO–acetic anhydride and by oxygen over platinum, the corresponding 6-toluene-*p*-sulphonyloxy-compound (684) was only oxidised by the former reagent.[519] Platinum-catalysed oxidation has also been used as a step in the synthesis of 2-deoxy-streptamine (see p. 166).

[663] J. L. Godman and D. Horton, *Carbohydrate Res.*, 1968, **6**, 229.
[664] A. Kampf, A. Felsenstein, and E. Dimant, *Carbohydrate Res.*, 1968, **6**, 220.
[665] K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, 1968, **101**, 4199.
[666] K. Heyns, E. Alpers, and J. Weyer, *Chem. Ber.*, 1968, **101**, 4209.



(681) $R^1 = OH$, $R^2 = H$
(682) $R^1 = H$, $R^2 = OH$



(683) R = H
(684) R = OTs

Other Oxidations

Full details are now available of the ruthenium tetroxide method of oxidising secondary alcohol groups. It was emphasised that the success of the method is critically dependent on the use of the correct form of the dioxide (which is first oxidised to the tetroxide), namely the hydrated form, probably $RuO_2,2H_2O$. The stoicheiometry of the reaction was investigated as well as the solvent medium. Axial and equatorial hydroxy-groups were oxidised with equal ease.[667] Use of the reagent in synthetic sequences has been described (see, for example, refs. 493 and 498).

A new mild oxidant, 1-chlorobenzotriazole, has been described for the oxidation of secondary and primary alcohol groups; this reagent could possibly be of use in carbohydrate chemistry.[668]

Reduction

Complex metal hydride reduction of a keto-group in aldulose and ketulose derivatives (generally prepared by DMSO-oxidation) has been widely used for the synthesis of the epimer of the starting alcohol (see, for example, refs. 14, 194, 515–517, and 628).

A series of dialkylboranes has been investigated to find the best for the reduction of acylated aldono-1,4-lactones to the acetylated furanoses. In spite of some disadvantages, bis(3-methyl-2-butyl)-borane (disiamylborane) was found to be the best of those tried. Attention was drawn to the need to prepare the reagent and not to use commercial material, which was found to be unsatisfactory.[541]

[667] P. J. Beynon, P. M. Collins, D. Gardiner, and W. G. Overend, *Carbohydrate Res.*, 1968, **6**, 431.
[668] C. W. Rees and R. C. Storr, *Chem. Comm.*, 1968, 1305.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

As in Volume 1 these two topics are treated together since the great majority of new stereochemical information has been obtained by n.m.r. methods. This Chapter does, however, contain a small amount of material concerned with only one aspect. It does not refer to all applications of spectroscopy to the determination of structural features of compounds—these are now too numerous for individual consideration—but mention is made of the papers considered to be of most general interest and value.

A general review of the methods available for determining conformations of carbohydrates has appeared,[669] and two others have been published on n.m.r. specifically. One covers a variety of aspects of importance in carbohydrate chemistry,[670] while the other pays particular attention to applications to deoxy-sugars.[671]

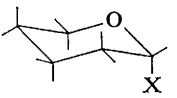
Pyranoid Systems

Quantitative Conformational Analysis.—Angyal has refined his earlier calculations of the relative free energies of the aldopyranoses in aqueous solution and, despite the acknowledged approximations used, has predicted the correct conformations of the various compounds, and has calculated the proportions of the α- and β-modifications present at equilibrium. Again his results are in good agreement with determined values.[24] An extension of the work led to the calculation of the position of equilibrium existing between free sugars and anhydro-modifications;[173] this is discussed more fully in Chapter 4. A related approach to the problem of aldopyranose conformations which however did not achieve such good correlation with experimental findings involved calculations of non-bonding interactions using Kitaigorodsky functions.[672] The *C*1-conformation was determined to have the lowest energy for all D-hexoses and for most D-pentoses; however α-D-arabinose and α-D-ribose were exceptional, and for β-D-arabinose and α-D-lyxose the energy difference between the two regular chairs is very

[669] S. Hirano, *Kagaku No Ryoiki*, 1968, **22**, 54.
[670] *Ann. Rev. N.M.R. Spectroscopy*, ed. E. J. Mooney, 1968, vol. 1.
[671] L. D. Hall and J. F. Manville, *Adv. Chem. Ser., No.* 74, 1968, 228.
[672] P. R. Sundararajan and V. S. R. Rao, *Tetrahedron*, 1968, **24**, 289.

small. The results were discussed with reference to early work by Reeves and Kelly.

Model Compounds.—Several observations made with non-carbohydrate models are of direct relevance; in particular, important contributions have been reported on the assessment of the anomeric effect. Eliel and Giza have published an important paper following their observations on the *cis-trans*-equilibrium of various 2-alkoxy and 2-alkylthio-tetrahydropyrans and 2-alkoxy-1,3-dioxanes. Their findings can be summarised as follows: (*i*) an RS group shows a somewhat smaller anomeric effect than the RO group; (*ii*) the anomeric effects in 2-alkoxy-4- and -6-methyltetrahydropyrans are slightly different, but the effect in 2-ethoxy-6-methyltetrahydropyran and 2,6-diethoxy-tetrahydropyran are very similar; (*iii*) the anomeric effect persists in 2-alkoxy-1,3-dioxanes. An authoritative discussion of the basis of the anomeric effect was given, and it was suggested that it could be assessed for any situation by consideration of the number of *syn*-axial electron lone-pairs on the acetal oxygen atoms. It was pointed out that the effect (for 2-hydroxy- and 2-alkoxy-tetrahydropyrans) decreases in magnitude with increase in the polarity of the solvent, and that this discounts an explanation of the basis of the effect which postulates overlap of the antibonding lobe of the axial C—X bond in compound (685) with the



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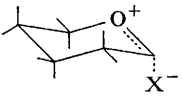

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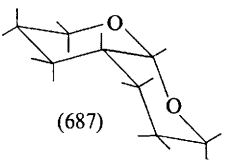

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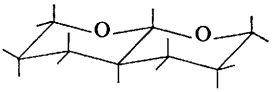


(688)

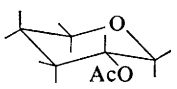
ring-oxygen unshared electrons. This requires an important contribution from the canonical form (686) which would be favoured by increase in the solvent polarity.[673]

Other workers have assessed the anomeric effect as lying within the range 1·3–2·8 kcal./mole for 2-alkoxytetrahydropyrans in non-polar solvents, the precise value depending upon the electron-withdrawing properties of the substituent.[674] With compound (687) which is thermodynamically more stable than the *trans*-isomer (688) the anomeric effect was determined as 1·4 kcal./mole.[675] The same value was obtained for the methoxy-group in

[673] E. L. Eliel and C. A. Giza, *J. Org. Chem.*, 1968, **33**, 3754.
[674] G. O. Pierson and O. A. Runquist, *J. Org. Chem.*, 1968, **33**, 2572.
[675] G. Descotes, M. Lissac, J. Delmau, and J. Duplan, *Compt. rend.*, 1968, **267***C*, 1240.

methanol from the system *cis*- and *trans*-1,3-dimethoxytetrahydropyran.[676] Anderson and Sepp have continued their related investigation of the anomeric effect using specifically substituted 2-alkoxytetrahydropyrans. The 2-methoxy-, 2-butoxy-, 2-hydroxy-, and 2-acetoxy-derivatives of 4-methyltetrahydropyran and of 6-hydroxymethyl- and 6-acetoxymethyl-tetrahydropyran were studied as model carbohydrates.[677] Similarly, 2-methoxy-*trans*-5,6-dimethyltetrahydropyran and its *cis*-isomer were examined and it was concluded that interaction occurs between substituents at C-5 and C-6 and causes ring flattening and alteration in C-1 effects, including the anomeric effect.[678]

The same authors have extended their studies to the consideration of the conformational requirements of substituents on other positions on tetrahydropyran rings, and have produced results of appreciable significance for carbohydrate chemistry. Examination of the equilibrium between the conformations (689) and (690) in different solvents showed that (689) was



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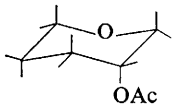


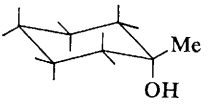
(690)

much less favoured than would have been expected on the basis of knowledge of substituted cyclohexanes, and may even be the less favoured form ($\Delta G = +0{\cdot}17 \rightarrow 0{\cdot}15$ kcal./mole). This is ascribed to a dipolar interaction between the acetoxy-group and the oxygenated portion of the ring. It is thus forcefully illustrated that dipolar effects other than those operating at C-1 (anomeric effect) in glycopyranosyl derivatives are important in governing molecular and conformational stabilities.[679] In related fashion, by carrying out equilibrations of 2-carbomethoxy-4-methyltetrahydropyrans, it was possible to ascertain the conformational free energy of the methyl group at this position on a tetrahydropyran ring (1·7 kcal./mole). Similarly, the values for the methyl group at C-5 and C-6 were found to be 1·27 and 1·70 kcal./mole, respectively. The preferences exhibited by the methyl group at positions 4 and 6 for the equatorial orientation are therefore very similar to the corresponding value for methyl on a cyclohexane ring, but a 5-methyl group suffers less interaction when axial. This is taken as evidence that the electrons on the ring oxygen atom offer less repulsion to the methyl group than do the hydrogens of a methylene group. The conformational preference of the carbomethoxy-group at position 2 for the equatorial orientation was 0·5 kcal./mole greater than the value for cyclohexane,

[676] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 1543.
[677] C. B. Anderson and D. T. Sepp, *Tetrahedron*, 1968, **24**, 1707.
[678] D. T. Sepp and C. B. Anderson, *Tetrahedron*, 1968, **24**, 6873.
[679] C. B. Anderson, D. T. Sepp, M. P. Geis, and A. A. Roberts, *Chem. and Ind.*, 1968, 1805.

which was accounted for by the presence of a reverse anomeric effect.[680] Hexuronosyl compounds will be subject to this factor.

Other workers have shown that the conformational free energies of methyl and hydroxy-groups attached geminally to a cyclohexane ring are non-additive. Whereas 1-methylcyclohexanol prefers the conformation (691) by 0·35 kcal./mole (DMSO and dioxan), the value calculated on the basis of additivity is *ca.* 1·0 kcal./mole.[506] Calculations of conformational energies of appropriate branched-chain pyranosyl compounds should therefore take this important deviation into account.



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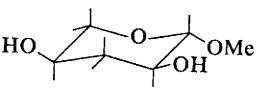

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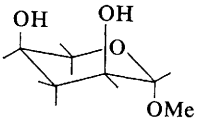

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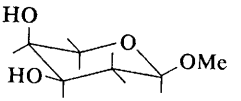


(694)

General Observations on Pyranoid Systems.—The important observation has been made that differences in optical rotations of carbohydrate derivatives measured in different solvents result directly from conformational changes. Thus, a plot of the rotation of methyl 3-deoxy-β-L-*erythro*-pentopyranoside *vs.* $J_{1,2}$, for values measured in a variety of solvents, was linear for six determinations (extreme values: D_2O, $J_{1,2} = 6$ Hz, $[\alpha]_D + 95°$; $CDCl_3$, $J_{1,2} = 2{\cdot}2$ Hz, $[\alpha]_D + 142°$). In water the compound existed largely in conformation (692), whereas in chloroform it was mainly in the alternative chair form (693). Calculated values for the optical rotations were in agreement with this. Similarly, methyl 2-deoxy-α-L-*erythro*-pentopyranoside exists largely as (694) in water, and as the other chair in chloroform, but the conformation of the β-anomer was largely independent of solvent.[681]

The true J values for cyclohexane have been measured and are of relevance in this area; from 1,1:2,2:3,3:4,4-octadeuteriocyclohexane the following values were obtained: $J_{1,1} = -13{\cdot}05$ Hz, $J_{1a,2a} = +13{\cdot}12$ Hz, $J_{1e,2e} = +2{\cdot}96$ Hz, and $J_{1a,2e} = +3{\cdot}65$ Hz. In particular it is of interest that the 1*e*,2*e* -and 1*e*,2*a*-values are not identical.[682]

A detailed paper has discussed the determination of relative signs of proton–proton couplings in carbohydrates. For saturated compounds vicinal constants were positive and geminal ones negative (in agreement with

[680] C. B. Anderson and D. T. Sepp, *J. Org. Chem.*, 1968, **33**, 3272.
[681] R. U. Lemieux and A. A. Pavia, *Canad. J. Chem.*, 1968, **46**, 1453.
[682] E. W. Garbisch and M. G. Griffith, *J. Amer. Chem. Soc.*, 1968, **90**, 6543.

the above); for unsaturated compounds both were positive. Coupling over four bonds was found to be positive for *e,e*-related protons and negative for *a,e*-protons in saturated systems. For unsaturated molecules all long-range couplings studied were found to be negative.[683]

A useful generalisation has been emphasised [170] for epoxides on pyranoid rings: $J = ca.\ 0$ for epoxide ring protons and their *trans*-neighbours, whereas such protons in the *cis*-relationship show $J = 2{\cdot}5$–$4{\cdot}5$ Hz. In the case of the aldehyde obtained on oxidation of 1,2:3,4-di-*O*-isopropylidene-D-galactose, attention has been drawn to the fact that $J_{5,6}$ was zero.[513]

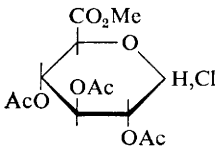
Observations of general interest have been made on the chemical shifts of pyranoid ring protons. The n.m.r. spectra of nine methyl glycopyranosidulose derivatives were compared in $CDCl_3$ and in C_6D_6, and it was observed that the anomeric protons all resonated at higher fields in benzene than in chloroform (+ve effect), but that the size of the solvent shift (0·1–0·6 p.p.m.) depended upon the position of the ketonic group on the ring. (How much it varied with anomeric configuration could not be assessed from the compounds selected.) Alternatively, the methoxy-protons suffered a uniform shift of *ca.* +0·5 p.p.m. The investigation revealed a probable further means of differentiating between *endo*- and *exo*-methyl groups of isopropylidene rings fused to pyranosiduloses since the former showed small solvent shifts, $< ca. \pm 0{\cdot}04$ p.p.m., whereas the latter showed effects of *ca.* +0·15 p.p.m.[684] In similar fashion the solvent shifts of acetoxy-protons have been measured for a number of C-1 acetylated carbohydrates. A difference for axial and equatorial groups was noted, and possible factors contributing to the effect were discussed.[685] A further detailed study has been made of the chemical shifts of axial and equatorial *O*- and *N*-acetyl groups of a wide variety of compounds in various solvents. The limitations in the use of these values in structural analysis were discussed.[686]

Chemical shifts of ring protons were considered after the observation was made that tetramethylammonium halides added to acetonitrile solutions of tetra-*O*-acetyl-β-D-glycopyranosyl halides and aryl tetra-*O*-acetyl-β-D-glucopyranosides caused specific deshielding of the axial protons of the 'lower' side of the pyranoid rings (1-H, 3-H, 5-H). The deshielding influences increased as the halide ion radius decreased. In addition the *ortho*-hydrogens of the phenyl groups were also deshielded. It was considered that the observation resulted from the formation of complexes of the ions with the undersides of the molecules as a whole, and that this approach may be used to detect electrophilic regions of molecules.[687]

Complexes with copper ions have also been detected by n.m.r. means.[284]

[683] L. D. Hall and J. F. Manville, *Carbohydrate Res.*, 1968, **8**, 295.
[684] R. F. Butterworth, M. H. Freemantle, and H. G. Overend, *Chem. and Ind.*, 1968, 1485.
[685] M. H. Freemantle and W. G. Overend, *Chem. Comm.*, 1968, 503.
[686] F. W. Lichtenthaler and P. Emig, *Carbohydrate Res.*, 1968, **7**, 121.
[687] J. S. Martin, J.-I. Hayami, and R. U. Lemieux, *Canad. J. Chem.*, 1968, **46**, 3263.

Specific Pyranoid Compounds.—Investigation of the anomeric chlorides (695) and (696) showed that the α-anomer adopted the *C*1-conformation,



(695) α-Anomer
(696) β-Anomer

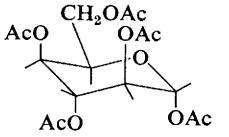
whereas the β-compound existed in some other ring form, presumably because of the influence of the anomeric effect (see Vol. 1, p. 84).[306] The spectra of a series of partially acylated methyl β-D-glucopyranosides having ester groups at C-6 have been compared, and various generalisations relating to the influence of substituents on the signals of 6-H were noted.[688] A study of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, its 3-ethyl ether, and the corresponding deacetylated glycosides in DMSO indicated that the chemical shifts of the hydroxyl protons of α-D-glucopyranosides varied in the order 1-OH (low field), 2-OH, 3-OH, 4-OH, and 6-OH, with the anomeric proton resonating between the first two. Previous results had indicated the reverse positions for the 2-OH and 4-OH resonances.[152] The spectra of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside and its 6,6′-deuteriated derivative have been interpreted in detail; partial virtual coupling in a four-spin system, exhibited by the compound, was discussed.[689]

In the course of work with mannans, the spectral features of several mannose derivatives, including mannobioses, have been investigated, and the influence of added borate to deuterium oxide solutions was noted.[690] Other α-D-mannopyranose compounds (and likewise rhamnose compounds) which have been examined are the adenine and theophylline nucleosides which exist in DMF, DMSO, and pyridine in the 1*C*-conformation with the aglycones equatorial.[651]

Coupling constants and chemical shifts of an extensive number of galactopyranose derivatives have been recorded and showed that the compounds exist predominantly in the *C*1-chair form.[691, 691a]

The 220 MHz n.m.r. spectra of α-D-idopyranose penta-acetate measured in deuteriated acetone and chloroform were completely first-order, and showed that the compound adopts the *C*1-conformation (697) in these solvents, contrary to expectations based on current ideas of quantitative

[688] A. P. Tulloch and A. Hill, *Canad. J. Chem.*, 1968, **46**, 2485.
[689] C. B. Barlow, E. O. Bishop, P. R. Carey, R. D. Guthrie, M. A. Jensen, and J. E. Lewis, *Tetrahedron*, 1968, **24**, 4517.
[690] P. A. Gorin, M. Mazurek, and J. F. T. Spencer, *Canad. J. Chem.*, 1968, **46**, 2305.
[691] H. Libert, I. Schuster, and L. Schmid, *Chem. Ber.*, 1968, **101**, 1902.
[691a] W. Sibral, H. Libert, and L. Schmid, *Monatsh.*, 1968, **99**, 884.



(697)

conformational analysis. This led to the speculation that in such systems forces other than the anomeric effect can stabilise ring forms having bulky axial groups. Attractions between 1,3-related axial groups or repulsion between 1,2-equatorial groups are thus indicated (but *cf.* 3-acetoxytetrahydropyran, p. 191). ^4J-Couplings (*ca.* 1 Hz) were observed between 1-H and 3-H, and between 2-H and 4-H as expected, but also 1-H was seen to be coupled to 4-H ($J_{1,4}$ 0·6 Hz).[692]

Several derivatives of *N*-acetylneuraminic acid, *e.g.* (315), were examined and shown to exist in the 1*C* conformation; in some instances long-range coupling was used to assign configuration at C-2.[353]

Furanoid Systems

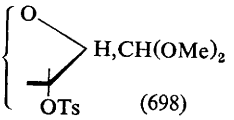
Detailed n.m.r. spectral studies have been carried out on an extensive series of pentofuranosyl derivatives, and have led to tentative assignments of the conformations adopted by the compounds in solution; the study represents the fullest survey and discussion of this topic so far carried out. It was pointed out that anomeric configurations can be assigned from the observation that 1-H is shielded by a *cis*-C-2-ring substituent. Similarly, *cis*-C-4-substituents shield 3-H.[693]

A related study was made of alkyl 3,5,6-tri-*O*-benzyl-D-glucofuranosides and their 2-esters[69] (see also ref. 59). A detailed examination has been made (at 100 MHz) of 1,2:3,5-di-*O*-benzylidene-α-D-glucofuranose and its 6-*O*-methyl, -acetyl, and -benzoyl derivatives and of 1,2-*O*-benzylidene-α-D-glucofuranose and its 3,5,6-tribenzoate. It was concluded that for the monoacetals the furanose ring was in the symmetrical twist conformation, and for the diacetals that the conformation was the non-symmetrical twist. In the dibenzylidene compounds the 3,5-acetal exists in the chair form having the carbohydrate C-6 axial.[694]

Several L-sorbofuranose derivatives were studied similarly: for methyl 1,3,4,6-tetra-*O*-acetyl-α-L-sorbofuranose the T_3^4-conformation was allocated whereas the β-anomer existed in the T_4^3-form. Also, derivatives with 2,3-*O*-isopropylidene or 4,6-*O*-benzylidene groups had rings locked in this same shape.[695]

[692] N. S. Bhacca, D. Horton, and H. Paulsen, *J. Org. Chem.*, 1968, **33**, 2484.
[693] J. D. Stevens and H. G. Fletcher jun., *J. Org. Chem.*, 1968, **33**, 1799.
[694] B. Coxon, *Carbohydrate Res.*, 1968, **8**, 125.
[695] T. Maeda, K. Tori, S. Satoh, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1968, **41**, 2495.

In furanoid systems having the partial structure (698) the methoxy-resonances showed a 15 Hz separation when the groups were in the *cis*-relationship and little or no such separations when they were *trans*.[294]



(698)

Intramolecular hydrogen bonding in *trans*-2-alkoxy-3-hydroxytetrahydrofurans in carbon tetrachloride solution was examined by i.r. methods, and the results were interpreted in terms of the conformation of the rings and so should have applicability in the study of certain furanosides.[696]

An interesting n.m.r. study of the changes of ribose ring conformations in mono- and di-nucleotides has led to the conclusion that in the dimers the bases are stacked, and a $J_{1',2'}$ temperature dependence indicated that above 60° the ribose ring alters shape and then adopts the same conformation as is present in the monomer.[652] The conformation of the furanose ring in 2′- and 3′-*C*-methyladenosines has been studied by n.m.r.[497, 498] The general method for determining the anomeric configuration of pyrimidine nucleosides, involving examination of the chemical shifts before and after reduction of the 5,6-double bonds, has been shown to be applicable also to glycosylindoles.[389]

A series of 2′- and 3′-halogenated nucleosides was examined, and by use of H,H- and H,F-couplings, various dihedral angles were determined and the furanosyl ring conformations were allocated. In this study correlations between electronegativities of ring substituents and coupling constants and chemical shifts were noted.[313] More specifically it was observed that in 5-fluoropyrimidine nucleosides spin–spin couplings are found between ^{19}F and the anomeric protons, the magnitudes being $> 1{\cdot}5$ Hz for β-isomers and $< 1{\cdot}5$ Hz for the α-anomers. The mechanisms of the couplings were discussed at length.[650]

Acyclic Systems

The n.m.r. spectra of seven aldose phenylosotriazoles have been examined in deuteriated DMSO, and chemical shifts and coupling constants have been interpreted in terms of preferred conformations of the flexible portions of the molecules. In keeping with generally-held views (although information on the conformations adopted by acyclic carbohydrates in solution is scant) zig-zag forms were found to be favoured except when these brought 1,3-related hydroxy-groups into eclipsed relationships[697] (see also ref. 698).

The structures of the chelate ring in osazones[360] and formazans[359] have been studied using ^{15}N n.m.r. techniques.

[696] W. W. Zajac jun., F. Sweet, and R. K. Brown, *Canad. J. Chem.*, 1968, **46**, 21.
[697] H. S. El Khadem, D. Horton, and T. F. Page jun., *J. Org. Chem.*, 1968, **33**, 734.
[698] G. G. Lyle and M. J. Piazza, *J. Org. Chem.*, 1968, **33**, 2478.

Amongst the many compounds whose conformations have been considered individually is the unusual 2,2′-anhydro-2-*C*-hydroxymethyl-1,3:4,6-*O*-isopropylidene-L-*xylo*-hexitol.[505]

Heteronuclear N.M.R. Studies

Reference to ^{15}N work is made above, and a brief communication has appeared on the examination of the $^{31}P,^{1}H$-coupling constants in the cyclic phenylphosphates of 1,2-*O*-isopropylidene-α-D-xylofuranose and methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside.[699]

Work with ^{19}F in nucleoside derivatives is mentioned on p. 196 but other more specific results have been reported. For the 2-halogeno-2-deoxy-D-glycopyranosyl fluorides described in ref. 308 the following relationships were noted: (*i*) the *vic*-$^{19}F,^{1}H$-coupling constants showed the same type of angular dependence as H,H-constants, so that $J_{gauche} < J_{a,a}$; (*ii*) with respect to a 2-deoxy-derivative, a C-2 halogen in a *gauche* relationship to 1-F shifts the ^{19}F resonance to a higher field, but when the halogens are in an antiparallel relationship the ^{19}F resonance is shifted to lower fields.[700] Study of other compounds has shown that steric requirements for four-bond couplings of ^{19}F and ^{1}H are similar to those for proton couplings, *i.e.* they are largest when interacting nuclei are both equatorial (in this case *ca.* 4 Hz). It was further shown that $^{4}J_{e,e}$ was positive in sign, whereas $^{4}J_{e,a}$ was negative. Several examples of $^{19}F,^{1}H$-couplings over five bonds were noted.[701]

Chlorofluoroacetates can be identified by a characteristic $^{19}F,^{1}H$-doublet.[214]

[699] L. D. Hall and R. B. Malcolm, *Chem. and Ind.*, 1968, 92.
[700] L. D. Hall and J. F. Manville, *Chem. Comm.*, 1968, 37.
[701] A. B. Foster, R. Hems, L. D. Hall, and J. F. Manville, *Chem. Comm.*, 1968, 158.

24
Other Physical Methods

1968 was notable for the increased use to which physical methods were put; mass spectrometry and *X*-ray crystallography were utilised more than ever before in the examination of carbohydrate derivatives.

Infrared Spectroscopy

The work reported on i.r. spectroscopy of carbohydrates emphasised the limitations of the method in the field; no new results of general significance were recorded.

Solid-phase spectra (in the O—H stretching region) of several simple carbohydrate compounds and their *O*-deuteriated derivatives have been investigated at −180°, at which temperature band resolution was found to be greatly improved. It was concluded that the observed fine structure arises from coupled O—H vibrations rather than from separated vibrations of individual hydroxy-groups.[702] Spectra of methyl β-D-xylopyranoside and its 1-thio-analogue have been studied as models for the repeating unit in naturally occurring xylans. Polarised spectra were examined and shifts on deuteriation were also considered.[703] Solid-state spectra of a variety of methyl ethers of D-glucose and D-xylose have been measured and considered in detail.[704] Other work relating to the examination of the intramolecular hydrogen bonding of hydroxy-groups may lead to a means for determining furanosyl ring conformations.[696]

From work on the i.r. spectra of cyclic acetals of ketoses it was claimed that the carbohydrate ring size was assignable from the position of a band in the region 680–725 cm.$^{-1}$, if the C-1 hydroxy-group is unsubstituted.[705] Furanosyl compounds were said to show an absorption band at 684 ± 4 cm.$^{-1}$, whereas in pyranosyl analogues this band occurred at 714 cm.$^{-1}$. On the basis of the evidence presented, however, this could be an unreliable generalisation.

Comments have been made on the use of i.r. spectroscopy in assigning the position of sulphate groups on carbohydrates,[265] and the technique has been used to determine the structures of *N*-aryl-glycosylamines.[386]

[702] A. J. Michell, *Austral. J. Chem.*, 1968, **21**, 1257.
[703] A. J. Michell, *Austral. J. Chem.*, 1968, **21**, 2451.
[704] A. J. Michell, *Tetrahedron*, 1968, **24**, 4021.
[705] J. R. Patil and J. L. Bose, *Carbohydrate Res.*, 1968, **7**, 405.

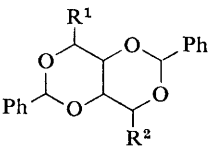
Mass Spectrometry

Much of the ground-work now having been done, mass spectrometry has moved into the category of an important analytical tool in carbohydrate chemistry, and many of the applications in 1968 were to analytical work rather than to compounds of known structure. The five products of pertrimethylsilylation of fructose were thus characterised,[166] and trimethylsilyl ethers were also used in the determination of the number and sites of attachment of methyl groups in carbohydrate ethers,[549, 706] in the identification of the substitution pattern of glyceryl lactates,[707] and, most significantly, in linkage analysis of oligosaccharides. Disaccharides linked $1 \rightarrow 2$, $1 \rightarrow 3$, $1 \rightarrow 4$, and $1 \rightarrow 6$ could all be distinguished, but the stereochemistry of the bonds could not be assigned; the positions of the inter-unit bonds in a trisaccharide were determined.[708] Trimethylsilyl ethers have been shown to make more polar nucleosides (guanine and cytidine) and nucleotides susceptible to examination by this technique.[653]

The mass spectra of methyl and some trideuteriomethyl ethers of alditols have been examined and the fragmentation mechanisms were discussed,[709] and methyl ester methyl glycosides of methylated uronic, aldobiuronic, and aldotriuronic acids have been similarly studied.[710]

A detailed paper appeared on the mass spectral fragmentation of peracetates of unsaturated sugars (four samples) and of anhydrodeoxyalditols. It was concluded that the results for the unsaturated derivatives were consistent with their being intermediates in the breakdown of hexose peracetates, and further that the stereochemistry of the anhydro-sugars was determinable by this method. One branched-chain compound was examined, from which it appeared that mass spectrometry could be used in the assignment of the position of branching in such compounds.[711]

Several acetals have been studied. It was shown that the molecular weights, and in some cases the stereochemistry, of isopropylidene alditols could be determined.[712] With the analogous benzylidene compounds a novel type of fragmentation was observed for the system (699). The



(699)

[706] G. Petersson and O. Samuelson, *Svensk Pappepstidn.*, 1968, **71**, 77.
[707] P. E. Brandt, N. Krog, J. B. Lauridsen, and O. Tolboe, *Acta Chem. Scand.*, 1968, **22**, 1691.
[708] N. K. Kochetkov, O. S. Chizhov, and N. V. Molodtsov, *Tetrahedron*, 1968, **24**, 5587.
[709] L. S. Golovkina, N. S. Wulfson, and O. S. Chizhov, *Zhur, org. Khim.*, 1968, **4**, 737.
[710] V. Kováčik, Š. Bauer, J. Rosík, and P. Kováč, *Carbohydrate Res.*, 1968, **8**, 282, 291.
[711] A. Rosenthal, *Carbohydrate Res.*, 1968, **8**, 61.
[712] N. S. Wulfson, O. S. Chizhov, and L. S. Golovkina, *Zhur. org. Khim.*, 1968, **4**, 744.

implications of this finding were discussed with particular reference to the fragmentation of methyl 4,6-*O*-benzylidene-hexopyranosides and their derivatives.[713] In another paper on benzylidene acetals the Russian workers showed how mass spectrometry can be of use in identifying structures and configurations. In particular the unusual fragmentation mentioned above was utilised.[714]

Unsubstituted compounds can also, under some circumstances, be subjected to examination. Thus, naturally occurring glycosides were found to cleave at C-1 so that the aglycone could be recognised, but, also, the presence of ester groups on the sugars could be detected, and their positions of attachment can conceivably be ascertained.[120] *C*-Glucosides have also been studied and from the fragmentation patterns the position of attachment could again be determined.[715] The spectra of a series of cyclohexane triols, tetrols, pentols, and hexols, and some of their deuteriated derivatives have been measured, and it was concluded that differences in peak intensities are sufficient to allow stereochemical deductions to be made.[716] The work follows that on cyclohexane diols [717] which indicated that positional and stereochemical isomers can be characterised.

Applications of g.l.c. combined with mass spectrometry are referred to on p. 206.

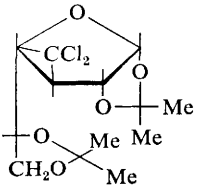
X-Ray Crystallography

Increasingly this technique is being used in structural analysis although compounds of known chemical composition are still commonly examined. In 1968 more reports on the crystallography of carbohydrate compounds appeared than in any single year previously.

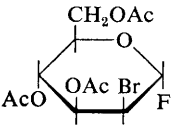
Compounds of uncertain structure which were fully characterised were (700) [718] (see Vol. 1, p. 129) and (701),[307] both obtained from reactions of unsaturated carbohydrates, (702),[412] prepared by an epoxide ring-opening reaction, and (703).[719] In addition, the assigned structures of streptomycin,[603] polyoxin C,[620] and kanamycin [591] have been confirmed by crystallographic means.

Amongst the compounds of known chemical structure which were examined were a series of nucleosides and derivatives (the primed figures in parentheses indicate the out-of-plane furanose ring atoms): thymidine (3′),[720] compound (704) (2′),[721] adenosine 3′,5′-cyclic phosphate (4′),[722]

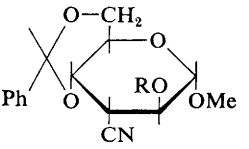
[713] O. S. Chizhov, L. S. Golovkina, and N. S. Wulfson, *Carbohydrate Res.*, 1968, **6**, 138.
[714] O. S. Chizhov, L. S. Golovkina, and N. S. Wulfson, *Carbohydrate Res.* 1968, **6**, 143.
[715] A. Prox, *Tetrahedron*, 1968, **24**, 3697.
[716] A. Buchs, E. Charollais, and T. Posternak, *Helv. Chim. Acta*, 1968, **51**, 695.
[717] A. Buchs, E. Charollais, and T. Posternak, *Helv. Chim. Acta*, 1968, **51**, 688.
[718] J. S. Brimacombe, P. A. Gent, and T. A. Hamor, *J. Chem. Soc.* (*B*), 1968, 1566.
[719] R. Hoge and J. Trotter, *J. Chem. Soc.* (*A*), 1968, 267.
[720] P. Tollin, H. R. Wilson, and D. W. Young, *Nature*, 1968, **217**, 1148.
[721] E. Shefter, *J. Pharm. Sci.*, 1968, **57**, 1157.
[722] K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, 1968, **159**, 206.



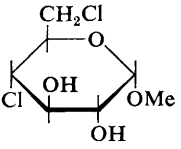
(700)



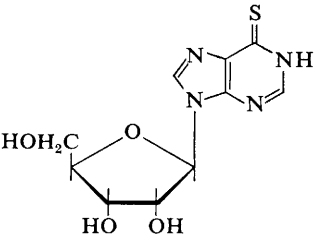
(701)



(702) R = *p*-Br·C_6H_4·SO_2



(703)



(704)

uridine 3′,5′-cyclic phosphate (3′),[723] and barium and disodium inosine 5′-phosphate (2′)[724].

Another furanosyl compound examined was methyl α-D-lyxofuranoside which had C-3 as the out-of-plane atom (E^3 conformation). This should be compared with the T_2^3 and E_3 conformations earlier proposed as likely for this molecule in solution. As has been found for pyranosyl compounds with equatorial C-1 groups, the C-1—1-O bond length was shorter than expected.[725] L-Ascorbic acid has been found to possess an almost planar ring.[726]

In the pyranose series β-D-glucopyranose and cellobiose have been re-examined and their structures refined,[727] and methyl α-D-glucopyranoside was found to exist in the expected C-1 conformation.[728] With all these compounds the C-1—1-O bonds were found to be shorter than expected.

Three crystalline modifications of D-mannitol have been examined, all of which possess a planar zig-zag carbon chain and differ in the arrangements of the intramolecular hydrogen bonds.[729, 730] D-Galactitol[731] and DL-arabinitol[732] also possess planar carbon chains, and in calcium α-D-glucoisosaccharinate, C-2′, C-2, C-3, and C-4 form such a zig-zag

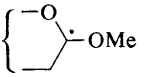
[723] C. L. Coulter, *Science*, 1968, **159**, 888.
[724] N. Nagashima and Y. Iitaka, *Acta Cryst.*, 1968, **B24**, 1136.
[725] P. Groth and H. Hammer, *Acta Chem. Scand.*, 1968, **22**, 2059.
[726] J. Hvoslef, *Acta Cryst.*, 1968, **B24**, 23.
[727] S. S. C. Chu and G. A. Jeffrey, *Acta Cryst.*, 1968, **B24**, 830.
[728] H. M. Berman and S. H. Kim, *Acta Cryst.*, 1968, **B24**, 897.
[729] H. M. Berman, G. A. Jeffrey, and R. D. Rosenstein, *Acta Cryst.*, 1968, **B24**, 442.
[730] H. S. Kim, G. A. Jeffrey, and R. D. Rosenstein, *Acta Cryst.*, 1968, **B24** ,1449.
[731] H. M. Berman and R. D. Rosenstein, *Acta Cryst.*, 1968, **B24**, 435.
[732] F. D. Hunter and R. D. Rosenstein, *Acta Cryst.*, 1968, **B24**, 1652.

conformation while the carboxylate group and C-5 project from opposite sides of the plane.[733]

Electron Spin Resonance Spectroscopy

Spectra of methyl α-D-glucopyranoside and of some of its tetra-*O*-aroyl esters have been studied, and for the unsubstituted glycoside the spectra were related to the radicals (705) and (706). For the esters, radicals (706)

(705)



(706)

were believed to be mainly responsible.[734] The spectrum derived from an irradiated single crystal of deoxyadenosine monohydrate has been examined and discussed.[654]

[733] R. Norrestam, P. E. Werner, and M. von Glehn, *Acta Chem. Scand.*, 1968, **22**, 1395.
[734] I. M. Sarkar, J. C. Arthur jun., and O. Hinojosa, *Carbohydrate Res.*, 1968, **6**, 333.

25
Polarimetry

Monochromatic Polarimetry

The interesting observation has been made that the differences in optical rotations exhibited by some carbohydrates in different solvents are due directly to conformational changes.[681]

In Part 1 of a series of papers on empirical correlation of rotations of glycosyl compounds, Yamana has demonstrated a linear relationship between molecular rotations of a series of acyl glycopyranosyl halides of any sugar and the atomic refraction of the halogen. He has now considered the free sugars, methyl and phenyl glycosides and peracetates of pyranoses, and estimated the functions (*S*) of the OH, OMe, and OPh groups which correspond to the atomic refraction (but which are quantitatively different from these). *S* Values plotted *vs.* molecular rotations gave linear relationships when: (*i*) the aglycone was an atom and the ring hydroxy-groups were acetylated or benzoylated; (*ii*) the aglycone was an axial methoxy-group and the ring substituents were OH or OAc; and (*iii*) when an axial OH or OPh was present at C-1 and the ring hydroxy-groups were unsubstituted.[734a] In a later paper of the series the influence of altering substituents at C-6 of glycopyranosyl derivatives was considered; plots of molecular rotation *vs.* *S* values were presented and generalisations were noted and discussed.[735] The same author has examined the relationships between the slopes of the plots of the molecular rotation *vs.* atomic refractions of the halides for acetylated aldopyranosyl halides in terms of Hudson's and Whiffen's (or Brewster's) methods, and the partial molar rotation method. The first two do not account for the observations whereas the last does quite satisfactorily.[736]

Optical Rotatory Dispersion and Circular Dichroism

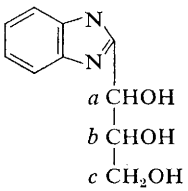
Appreciable developments in this area of research were recorded in 1968.

The method of structural determination of α-diols and α-amino-alcohols based on cuprammonium complexing has been significantly extended by

[734a] S. Yamana, *J. Org. Chem.*, 1968, **33**, 185.
[735] S. Yamana, *J. Org. Chem.*, 1968, **33**, 1819.
[736] S. Yamana, *Tetrahedron*, 1968, 1559.

measuring circular dichroism spectra rather than rotations at one wavelength; the work conclusively established that vicinal diols or amino-alcohols in which the dihedral angle is 180° do not complex with the reagent.[737] C.d. curves for various simple carbohydrates have been measured, and for free sugars the first curve below 200 mμ has been observed. It was concluded that anomeric configurations of glycosides do not determine the sign of this first band which is controlled largely by the configurations at C-4.[738] The same group of workers extended their studies on the o.r.d. of carbohydrates to an examination of the methyl glycosides of uronic acids and 2-acetamido-2-deoxy-sugars. Difference curves set up with reference to those of the glycosides revealed that for uronic acids there is an extremum near 220 mμ with a lower one below 190 mμ, and for the amido-derivatives the extrema (associated with the optically-active carbonyl group) are at 220 and 200 mμ.[739] It was claimed that acetylated phenyl D-glycosides exhibit multiple Cotton effects in the 260–274 mμ region which are positive for α-compounds and negative for the anomers;[740] more compounds need to be examined before this becomes a generalisation.

A considerable proportion of the reports relate to nitrogenous derivatives. O.r.d. data have been presented for a series of *N*-salicylidene derivatives of amino-sugars and of their metal chelates and also of *N*-pyridoxaline compounds and their chelates.[741] The c.d. curves of twenty-eight azido-sugars were recorded and application of the azide octant rule was discussed in detail.[398] From o.r.d. studies of heterocyclic compounds derived from sugars it is becoming evident that configuration can be determined at asymmetric centres adjacent to the chromophores and also at more remote centres; a study of polyhydroxyalkyl-benzimidazoles (707) and -quinoxalines (708) was reported. A D-centre at position *a* gives rise to positive



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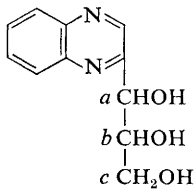


(708)

o.r.d. curves but the amplitude of the Cotton effect is governed by the *a*, *b* steric relationship, *threo*-compounds giving higher amplitudes and having higher rotational values at long wavelengths. Furthermore, position *c* influences rotations, *arabino*-derivatives giving higher rotational values

[737] S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Chem. Comm.*, 1968, 1580.
[738] I. Listowsky and S. Englard, *Biochem. Biophys. Res. Comm.*, 1968, **30**, 329.
[739] I. Listowsky, G. Avigad, and S. Englard, *Carbohydrate Res.*, 1968, **8**, 205.
[740] T. Sticzay, C. Peciar, and Š. Bauer, *Tetrahedron Letters*, 1968, 2407.
[741] M. Maeda, T. Kinoshita, and A. Tsuji, *Tetrahedron Letters*, 1968, 3407.

than *xylo-* which had higher values than *ribo-* or *lyxo-*. A method of potential value for determining configurations at positions 3, 4, and 5 of monosaccharides is therefore provided.[742] A related study of phenylosotriazoles provided the following generalisation: a positive Cotton effect at 265 mμ is diagnostic of the D-configuration at C-3. Conformations of the flexible side-chains in these compounds and their acetates were determined by n.m.r. and a sector rule applicable to the series was devised.[698]

The c.d. curve of L-ascorbic acid was negative (240 mμ minimum) in *M*-hydrochloric acid and positive at this wavelength at pH 6. This was interpreted as indicating that the acid itself and an anion formed by loss of a proton from 3-*O* have different conformations. It was claimed that this interpretation is supported by n.m.r. and *X*-ray evidence.[743] O.r.d. curves of several tetruloses and 2- and 3-pentuloses have been studied in aqueous solution. Those which cannot form an internal hemiacetal showed much stronger curves, and the sign of the Cotton effects were determined by the chirality of the atom(s) adjacent to the carbonyl group (negative for *R* centres and *vice versa*).[744]

An important fundamental study of the o.r.d. and c.d. curves of a variety of nucleosides, including compounds with double bonds in the sugar moiety and substituted nucleosides, has been reported, and the results were correlated with expectations from theoretical considerations. The approach represents a significant advance on the empirical methods previously applied in this area.[745] The same authors have discussed the c.d. curves of 3,5-cyclopurine nucleosides in detail,[746] and other workers have reported the o.r.d. spectra of many nucleosides (including anomeric pairs) and their 5′-phosphates.[747]

The first magnetic circular dichroism studies of nucleosides and their component bases have been carried out, and the technique was shown to be of value in distinguishing between purine and pyrimidine derivatives (particularly useful for small quantities of material) and for resolving overlapping absorption bands. The technique does not offer means for obtaining conformational information on optically active molecules.[748]

[742] W. S. Chilton and R. C. Krahn, *J. Amer. Chem. Soc.*, 1968, **90**, 1318.
[743] G. C. Kresheck, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 374.
[744] T. Sticzay, C. Peciar, K. Babor, M. Fedoroňko, and K. Linek, *Carbohydrate Res.*, 1968, **6**, 418.
[745] D. W. Miles, S. J. Hahn, R. K. Robins, M. J. Robins, and H. Eyring, *J. Phys. Chem.*, 1968, **72**, 1483.
[746] D. W. Miles, R. K. Robins, and H. Eyring, *J. Phys. Chem.*, 1967, **71**, 3931.
[747] T. Nishimura, B. Shimizu, and I. Iwai, *Biochim. Biophys. Acta*, 1968, **157**, 221.
[748] W. Voelter, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem, Soc.*, 1968, **90**, 6163.

26
Separatory and Analytical Methods

As in Volume 1, separatory techniques will be dealt with in turn and will be followed by reports of direct analytical methods. In general, extensive cross-referencing is not employed between this Chapter and others.

Chromatographic Methods

Gas–Liquid Chromatography.—In this section reports on aspects of general significance will be treated first; specific application follows.

The use of a g.l. chromatograph (capillary columns) in separations of diastereoisomeric aldonate esters and consequent determination of configuration at C-2 of the constituent acids has been referred to earlier,[531] and for the first time separations of ^{1}H and ^{2}H TMS derivatives of carbohydrates have been investigated.[748a] Recoveries of TMS ethers of free sugars from gas chromatographs were determined using ^{14}C-labelled sugars and scintillation counting techniques. These varied somewhat for different sugars and were of the order of 30%. Decomposition of the materials and adsorption of the products throughout the length of the column were held to be responsible for these low values.[749] Products of the direct pyrolysis of glucose were found to contain more than 100 components by g.l.c.[34] In other work, products of related degradation of free sugars were studied by combined g.l.c.–mass spectrometry procedures,[33] and the pertrimethylsilylated fructoses were examined similarly.[166] In a further application to free sugars g.l.c. was used (TMS ethers) to determine the equilibrium composition of D-galactose in pyridine. The products were separated in preparative work and examined by n.m.r.[20]

A series of reports on the g.l.c. of alditol derivatives (mainly TMS ethers) has appeared as a consequence of the value of these compounds in free-sugar analysis. Thus, for example, the components of starch syrup have been analysed following a reduction procedure; in this way the problem of multiple peaks deriving from single sugars is avoided.[750] Other workers have adopted similar approaches,[751] and the general procedures

[748a] N. C. Saha and C. C. Sweeley, *Analyt. Chem.*, 1968, **40**, 1628.
[749] E. F. Jansen and N. C. Baglan, *J. Chromatog.*, 1968, **38**, 18.
[750] T. Cayle, F. Viebrock, and J. Schiaffino, *Cereal Chem.*, 1968, **45**, 154.
[751] G. G. S. Dutton, K. B. Gibney, G. D. Jensen, and P. E. Reid, *J. Chromatog.*, 1968, **36**, 152.

have been applied to glycosylalditols,[752] naturally occurring alditols,[753] trifluoroacetylated alditols,[754] polyol acetates derived from the neutral sugar components of glycoproteins,[755] and polyol acetates [756] and trifluoroacetates [757] derived from 2-amino-2-deoxyhexoses. A g.l.c. method has also been developed for the analysis of mono- and di-saccharides in urine.[758]

More specifically, twenty-two methylated methyl galactosides have been examined as have TMS and methyl ethers of glucuronides.[759] A method based on g.l.c. of TMS derivatives of *N*-acetylneuraminic acid is reported to be more accurate and selective than previous procedures for determining sialic acids in biological fluids.[760]

G.l.c. methods have been developed for the quantitative analysis of the bases, nucleosides, and nucleotides obtained from nucleic acid hydrolysates. Full details are recorded of all aspects of the analytical procedures in a comprehensive survey of the problems involved.[761] A related report has described dimethylsilyl, triethylsilyl, tri-*n*-butylsilyl, and triphenylsilyl derivatives of adenosine; silylation of nucleotides and coenzymes was also reported.[762]

Two methods based on g.l.c. of TMS derivatives have been described for the determination of lincomycin in various antibiotic preparations; the results compared favourably with those obtained by microbiological assay methods.[618, 619]

Column Chromatography (Including Ion-exchange Chromatography).—Gel filtration has been employed to separate glucose, sucrose, and phenolic glycosides,[763] and the resolving powers of various clays with respect to methylated methyl glycosides were compared with various spectral features, but little correlation was observed.[764]

A series of reports has appeared on the use of ion-exchange resins in the separation of carbohydrates and their derivatives. An automated method for determining free sugars and alditols was developed based on the use of strong-base resins in the sulphate form. Analysis was carried out by automatic colorimetric determination of the sugars and of the total carbohydrate

[752] G. G. S. Dutton and A. M. Unrau, *J. Chromatog.*, 1968, **36**, 283.
[753] N. L. Gregory, *J. Chromatog.*, 1968, **36**, 342.
[754] M. Matsui, M. Okada, T. Imanari, and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1383.
[755] W. F. Lehnhardt and R. J. Winzler, *J. Chromatog.*, 1968, **34**, 471.
[756] M. B. Perry and A. C. Webb, *Canad. J. Biochem.*, 1968, **46**, 1163.
[757] Z. Tamura, T. Imanari, and Y. Arakawa, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 1864.
[758] T. Bhatti and J. R. Clamp, *Clinica Chim. Acta*, 1968, **22**, 563.
[759] Yu. S. Ovodov and A. F. Pavlenko, *J. Chromatog.*, 1968, **36**, 535.
[760] D. A. Craven and C. W. Gehrke, *J. Chromatog.*, 1968, **37**, 414.
[761] C. W. Gehrke and C. D. Ruyle, *J. Chromatog.*, 1968, **38**, 473.
[762] R. L. Hancock, *J. Gas Chromatog.*, 1968, **6**, 431.
[763] A. Repaš and B. Nikolin, *J. Chromatog.*, 1968, **35**, 99.
[764] V. T. Bykov, A. A. Vas'kovskaya, and A. I. Chernitsyn, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2636.

using a split-stream system.[765] However, care must be taken in the use of resins for such purposes since it has been shown that a reversible reaction occurs between D-xylose and Amberlite resin IR-45 (OH) producing a Schiff's base or a glycosylamine.[766] Separation of free sugars on anion resins in the sulphate form and on cation resins in the lithium form have been surveyed; mixtures containing components difficult to resolve on one type of resin can be handled effectively by application of each in turn.[767] Dowex 50WX4 was found, however, to be more efficient for such separations when in the potassium rather than the lithium or barium forms.[768]

Two papers have described the use of resin chromatography for separating hexosamines obtained from natural products,[769, 770] and other papers report similar applications to glycosides[771] and uronic acids and uronic acid-containing oligosaccharides.[772] The problems involved in the separation of anomeric glycosyl phosphates have been discussed, and the use of resin chromatography was described.[773] In other work the resolution of nucleoside and nucleotide mixtures on cation resins using ammonium formate buffers was reported.[774]

Paper Chromatography.—Empirically derived equations have been proposed for the calculation of paper chromatographic mobilities of hexoses and 6-deoxyhexoses which gave good agreement with observed values.[775] However, this treatment must be considered to be over-simplified as it ignores the conformational features of the sugars which are undoubtedly as important as configuration in determining chromatographic characteristics.

Methods have been developed for the precipation of the barium salts of sugar phosphates important in the pentose phosphate cycle. Barium was removed and the phosphates were chromatographed on paper as their sodium salts. Mobilities of these salts and of the parent sugars were reported.[776] A series of paper-chromatography solvents based on acetonitrile and aqueous buffers was described for use with various nucleosides, nucleotides, and the nucleic acid bases.[777]

Twelve reagents used for detecting free sugars on paper chromatograms were assessed for specificity and sensitivity especially with respect to sugars found in glycoprotein hydrolysates. 2,3,5-Triphenyl tetrazolium chloride and blue tetrazolium were the most sensitive while the Elson–Morgan and

[765] O. Samuelson and H. Strömberg, *J. Food Sci.*, 1968, **33**, 309.
[766] P. J. Murphy, G. N. Richards, and E. Senogles, *Carbohydrate Res.*, 1968, **7**, 460.
[767] O. Samuelson and H. Strömberg, *Acta. Chem Scand.*, 1968, **22**, 1252.
[768] R. M. Saunders, *Carbohydrate Res.*, 1968, **7**, 76.
[769] A. S. R. Donald, *J. Chromatog.*, 1968, **35**, 106.
[770] P. C. Kelleher and C. J. Smith, *J. Chromatog.*, 1968, **34**, 7.
[771] M. E. Evans, L. Long jun., and F. W. Parrish, *J. Chromatog.*, 1968, **32**, 602.
[772] L.-A. Fransson, L. Rodén, and M. L. Spach, *Analyt. Biochem.*, 1968, **23**, 317.
[773] D. L. MacDonald, *Carbohydrate Res.*, 1968, **6**, 376.
[774] E. W. Busch, *J. Chromatog.*, 1968, **37**, 518.
[775] H. Amato, *J. Chromatog.*, 1968, **33**, 500.
[776] T. Wood, *J. Chromatog.*, 1968, **35**, 352.
[777] T. F. Gabriel, *J. Chromatog.*, 1968, **36**, 518.

naphthoresorcinol reagents had the best combined sensitivity and specificity for 2-amino-2-deoxy-compounds and uronic acids, respectively.[778] Azulene in dilute sulphuric acid is reported as a new spray reagent; the colours given by various sugars and the detection limits were recorded and compared with those given by other reagents.[779] A further reagent recommended for its potential value in quantitative work is based on a methanolic solution of malonic acid and aniline.[780]

Thin-layer Chromatography.—A new technique for preparative t.l.c. which avoids the requirement of removing a zone of absorbent has been reported; required areas are extracted by side-ways elution.[781] Trimethylsilyl ethers have been shown to be sufficiently stable to be subjected to t.l.c. separation.[165] A spray reagent based on aminoguanidine sulphate and chromic acid has been developed for the detection of free sugars; the detection limits are 0·1 μg and a wide range of sugars and glycosides and acetals were shown up. Fucose and galacturonic acid were observed to give characteristic colour tests.[782] A previously reported anthrone test for ketoses was found not to be specific as had been claimed; minor changes in the procedure were recommended to aid specificity.[783] A picric acid spray reagent has been recommended for the detection of epoxides, and although carbohydrate epoxides were not specifically investigated it appears likely that this method would be useful for detecting these compounds.[784]

A thorough investigation of the separation of free sugars was carried out using 20 carbohydrates and 42 solvent systems; this probably represents the most complete survey of free sugar t.l.c. so far reported.[785] Other workers developed a simple technique for routine analysis of sugars in biological fluids,[786] and others have described specifically the assay of radioactive compounds.[787] Circular t.l.c. was recommended for the rapid separation of free sugars (eight compounds resolved in 3 min.).[788]

Systems for the separation of glucose, galactose, 2-amino-2-deoxy-D-glucose (and -galactose), and their *N*-acetyl derivatives and neuraminic acid were described,[789] and several reports have dealt with the t.l.c. of oligosaccharides: the maltose homologues up to the nonasaccharide,[790] the

[778] J. Mes and L. Kamm, *J. Chromatog.*, 1968, **38**, 120.
[779] C. R. Engel and E. Sawicki, *Microchem. J.*, 1968, **13**, 202.
[780] H. Zentner, *Chem. and Ind.*, 1968, 1836.
[781] M. H. Stutz, W. D. Ludemann, and S. Sass, *Analyt. Chem.*, 1968, **40**, 258.
[782] P. M. Martins and Y. P. Dick, *J. Chromatog.*, 1968, **32**, 188.
[783] T. Koyama, Y. Kinura, Y. Takahashi, and R. Sawamura, *J. Pharm. Sci.*, 1968, **88**, 1090.
[784] J. A. Fioriti and R. J. Sims, *J. Chromatog.*, 1968, **32**, 761.
[785] M. Lato, B. Brunelli, G. Ciuffini, and T. Mezzetti, *J. Chromatog.*, 1968, **34**, 26.
[786] M. Lato, B. Brunelli, G. Ciuffini, and T. Mezzetti, *J. Chromatog.*, 1968, **36**, 191.
[787] A. E. Gal, *J. Chromatog.*, 1968, **34**, 266.
[788] M. H. Hashmi, N. A. Chughtai, and M. A. Shahid, *Mikrochim. Acta*, 1968, 679.
[789] A. E. Gal, *Analyt. Biochem.*, 1968, **24**, 452.
[790] V. A. de Stefanis and J. G. Ponte jun., *J. Chromatog.*, 1968, **34**, 116.

β-D-fructofuranosyl-sucroses,[791] the cellobiose homologues,[792] and the oligosaccharide constituents of cocoa beans.[793]

A wide variety of substituted glycosides was studied [794] and difficulties encountered in the detection of small quantities of cyanoglycosides have been overcome.[795]

Partial methylation of sugars to give methylated glycosides was recommended as a method for characterising sugars since the mixed products give characteristic patterns on t.l.c.[796] The differing complexing affinities of Pb^{2+} in alkali with polyols allowed various compounds to be readily separated on silica gel impregnated with lead nitrate.[797] A new method for determining ascorbic acid in biological fluids depends upon a t.l.c. separation followed by a complexiметric titration.[798]

Other Analytical Methods

In the area of general methods for free sugars, modifications have been recommended for the sulphuric acid procedure [799] and for the alkaline ferricyanide technique.[800] In the latter case 2,4,6-tripyridyl-*S*-triazine was used and it was claimed that 2 μmole of sugar can be determined accurately. Perchloric acid, sometimes used for the extraction of starch, can interfere with the anthrone method for determining carbohydrates.[801]

Specifically, enzymic procedures have been reported for the determination of glucose in biological fluids.[802, 802a] Automated methods of analysis have been developed.[803, 803a] Radioisotope dilution procedures have been used to determine the mannose, galactose, and fucose contents of glycoproteins,[804] and procedures for the quantitative determination of D-fructose in the presence of a large excess of D-glucose have been described.[805] Also, it has been reported that sucrose may be determined in the presence of glucose and fructose after destroying the reducing compounds with alkali.[806] In a different type of analysis the activity at each carbon of ^{14}C-labelled

[791] H. Hammer, *Acta Chem. Scand.*, 1968, **22**, 197.
[792] S. Saif-ur-Rahman, C. R. Krishnamurti, and W. D. Kitts, *J. Chromatog.*, 1968, **38**, 400.
[793] C. V. Pasupathy and R. O. B. Wijesekera, *J. Chromatog.*, 1968, **35**, 117.
[794] R. E. Wing, C. L. Collins, and J. N. BeMiller, *J. Chromatog.*, 1968, **32**, 303.
[795] L. D. Bennett and B. A. Tapper, *J. Chromatog.*, 1968, **34**, 428.
[796] V. E. Vaskovsky, R. G. Ovodova, Yu. S. Ovodov, V. T. Bykov, and A. A. Vaskovskaya, *Carbohydrate Res.*, 1968, **7**, 490.
[797] V. de Simone and M. Vicedomini, *J. Chromatog.*, 1968, **37**, 538.
[798] S. Baczyk and L. Duczmal, *Mikrochim. Acta*, 1968, 1291.
[799] M. J. Houle, R. L. Powell, and P. Fintschenko, *Analyt. Biochem.*, 1967, **21**, 462.
[800] G. Avigad, *Carbohydrate Res.*, 1968, **7**, 94.
[801] E. P. Bachelard, *Analyt. Chim. Acta*, 1968, **42**, 173.
[802] A. H. Kadish, R. L. Litle, and J. C. Sternberg, *Clinical Chem.*, 1968, **14**, 116.
[802a] A. G. Ware and E. P. Marbach, *Clinical Chem.*, 1968, **14**, 548.
[803] R. H. Laessig and B. J. Basteyns, *Microchem. J.*, 1968, **13**, 418.
[803a] R. J. Jolley and M. L. Freman, *Clinical Chem.*, 1968, **14**, 538.
[804] E. R. B. Grahan and A. Neuberger, *Biochem. J.*, 1968, **106**, 593.
[805] M. Nakamura, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 412, 417, 689, 696, 701.
[806] E. van Handel, *Analyt. Biochem.*, 1968, **22**, 280.

aldopentoses and aldohexoses may be determined using methods based on periodate degradation of the methyl glycosides.[807]

In the area of 2-amino-2-deoxyhexoses, publications have appeared on colorimetric analytical methods based on acetylacetone,[808] *p*-nitrobenzaldehyde followed by tetraethyl ammonium hydroxide,[809] and the Elson-Morgan procedure.[810] Alternatively, radioisotope dilution methods have been applied and the advantage of this approach has been discussed.[811] In all cases the techniques were developed to aid the analysis of biological material. In clinical work the determination of protein-bound hexosamines in sera of patients with amyloidosis showed high values but the variations found were such that analyses of this kind were concluded to be of no value for differentiating between several diseases.[812]

A method has been developed for the analysis of D-gluc-, D-galact-, and D-mann-uronic acids in mixtures using the carbazole colorimetric procedure,[813] and an enzyme method for determining the second of these acids has been reported.[814] The problem of lactonisation of uronic acids during the hydrolysis of acidic polysaccharides and its effect on their analyses has been discussed, and procedures for overcoming potential difficulties were described.[815]

In the area of esters, a specific enzymic assay of D-fructose 1-phosphate has been developed,[816] as has a micro-method (based on flame photometry) for the quantitative determination of sulphate groups.[817]

A general study was made of the micro-methods available in the study of the periodate oxidation of carbohydrates; periodate reduction, formic acid and formaldehyde release were all considered, and the identification of degradation products by g.l.c., polarography, and spectrophotometry was discussed. Deoxyaldoses and deoxyketoses were examined in particular.[818] More especially, a method for determining small concentrations of periodate has been developed which could offer advantages over the direct spectrophotometric method. 1,2-(*p*-Dimethylaminophenyl)ethane-1,2-diol (709) was used and its product of oxidation was determined spectrophotometrically at 352 mμ.[819] A procedure for the consecutive titration of iodate and

[807] P. L. Russ and R. D. Bevill, *Analyt. Biochem.*, 1968, **23**, 13.
[808] D. E. S. Stewart-Tull, *Biochem. J.*, 1968, **109**, 13.
[809] A. Nakamura, M. Maeda, K. Ikeguchi, T. Kinoshita, and A. Tsuji, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 184.
[810] J. J. Ludowieg and J. D. Benmaman, *Carbohydrate Res.*, 1968, **8**, 185.
[811] E. R. B. Graham and A. Neuberger, *Biochem. J.*, 1968, **109**, 645.
[812] L. Ruinen, J. H. Scholten, and E. Mandema, *Clinica Chim. Acta*, 1968, **19**, 49.
[813] C. A. Knutson and A. Jeanes, *Analyt. Biochem.*, 1968, **24**, 482.
[814] C. W. Nagel and S. Hasegawa, *Analyt. Biochem.*, 1967, **21**, 411.
[815] J. D. Blake and G. N. Richards, *Carbohydrate Res.*, 1968, **8**, 275.
[816] F. Heinz, *Hoppe-Seyler's Z. physiol. Chem.*, 1968, **349**, 859.
[817] S. A. Barker, J. F. Kennedy, P. J. Somers, and M. Stacey, *Carbohydrate Res.*, 1968, **7**, 361.
[818] L. I. Kudryashov, M. A. Chlenov, P. N. Smirnov, and S. D. Kovacheva, *Zhur. obshchei Khim.*, 1968, **38**, 74.
[819] R. Fields and H. B. F. Dixon, *Biochem. J.*, 1968, **108**, 883.

$$\begin{matrix} Me_2N \cdot C_6H_4 \cdot CHOH \\ | \\ Me_2N \cdot C_6H_4 \cdot CHOH \end{matrix}$$

(709)

periodate has been described which should be applicable in oxidation work with carbohydrates,[820] and a method based on the determination of formaldehyde liberated on periodate oxidation of free sugars has been applied to the analysis of the latter in biochemical systems. It has the advantage of being readily automated and of allowing the determination of 6-deoxyhexoses in the presence of other monosaccharides.[821]

During standard treatment with alkali (a procedure used in the analysis of nucleotides of RNA) decomposition of nucleotides occurs, and an investigation has revealed the correction factors which should be applied to the results obtained.[822]

[820] R. Belcher and A. Townshend, *Analyt. Chim. Acta*, 1968, **41**, 395.
[821] G. M. Brearley and J. B. Weiss, *Biochem. J.*, 1968, **110**, 413.
[822] J. Ahonen and E. Kulonen, *Acta Chem. Scand.*, 1968, **22**, 360.

27
Alditols

D-Mannitol was found to be an important constituent of celery petioles and is believed to contribute largely to the flavour.[823] Several papers have appeared on crystallographic studies (see Chapter 24). Allitol has been oxidised to L-allulose,[824] and L-*glycero*-D-*galacto*-heptitol prepared by reduction of the aldose.[10] 1,3-Dideoxy-D-*ribo*-hexitol and its L-*lyxo*-isomer have been synthesised,[540] as has the novel cyclopropyl derivative (556).[511]

1,2:5,6-Di-*O*-bromoethylidene-D-mannitol has been described.[185] The following new derivatives of D-arabinitol have been prepared:[198] the 1,5-di-*O*-trityl compound and its triacetate and tribenzoate; the 1,5-di-*O*-toluene-*p*-sulphonyl ester and its tribenzoate and tri-*O*-methyl ether; and the 1,5-dideoxy-1,5-di-iodo tribenzoate. Several papers have appeared on phosphates and phosphites of D-mannitol.[242, 250, 251] 1,2,4,6-Tetra-*O*-benzoyl-D-glucitol has been described.[230]

2,3:4,5-Dianhydro-D-iditol[180] and 2,5:3,6-di-anhydro-D-glucitol[825] have been synthesised. Several 2-amino-2-deoxyalditols have been prepared.[333, 334] The mass spectra of several isopropylidene-alditols have been investigated.[712]

[823] R. Becker, *J. Food Sci.*, 1968, **33**, 128.
[824] J. G. Carr, R. A. Coggins, L. Hough, B. E. Stacey, and G. C. Whiting, *Phytochemistry*, 1968, **7**, 1.
[825] L. Vargha and J. Kuszmann, *Carbohydrate Res.*, 1968, **8**, 157.

Part II

MACROMOLECULES

By

M. J. How
P. J. Somers

1
Introduction

The objectives stated in the Introduction to Volume 1 of this Series have been pursued in preparing Volume 2. Thus, the authors have attempted to summarise the information, published during 1968, on the chemistry of macromolecules which contain carbohydrate. Literature published during 1967 that was not available to, or was overlooked by, the author, before publication of Volume 1, has been included in Volume 2 in an attempt to provide, in the Series as a whole, a comprehensive record of the relevant literature from January 1967. In order to facilitate the retrieval of information published on a given topic since that date the same general arrangement of chapters used in Volume 1 has been continued in Volume 2.

2
General Methods

Analysis*

A further modification of the carbazole–sulphuric acid reaction of hexuronic acids gave increased sensitivity for galacturonic, mannuronic, and guluronic acids but decreased that of glucuronic acid.[1] Use of four different reaction conditions permitted the determination of mixtures of three hexuronic acids, the method being tested by the analysis of alginates.[1] Determination of the formaldehyde released on oxidation with periodate by reaction with acetylacetone provided a method for the quantitative measurement of monosaccharides, amino-sugars, and *N*-acetylneuraminic acid in the presence of other sugars.[2] A spectrophotometric method has been reported for the direct quantitative determination of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and muramic acid.[3] The anthrone–sulphuric acid reaction has been employed for the microdetermination of dextran in body fluids.[4] The relative sensitivity of various reagents for the detection and differentiation of sugars and sugar derivatives in glycoproteins has been investigated.[5] A method for the determination of starch and soluble carbohydrates in breakfast cereals and roughages has been reported[6] and its reliability established in a collaborative study.[7] Automated methods have been described for the determination of glucose by reaction with *o*-toluidine,[8] and blood serum sialic acids by reaction with 2-thiobarbituric acid.[9]

N-Acetylneuraminic acid has been quantitatively determined by g.l.c. after silylation in acetonitrile, using *trans*-stilbene[10] as internal standard. Borohydride reduction, followed by acetylation with acetic anhydride–sodium acetate at 135° for 2 hr. and analysis by g.l.c., provided a method

[1] C. A. Knutson and A. Jeanes, *Analyt. Biochem.*, 1968, **24**, 470, 482.
[2] G. M. Brearley and J. B. Weiss, *Biochem. J.*, 1968, **110**, 413.
[3] D. E. S. Steward-Tull, *Biochem. J.*, 1968, **109**, 13.
[4] W. Appel, V. Wirmer, and D. Sprengard, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 452.
[5] J. Mes and L. Kamm, *J. Chromatog.*, 1968, **38**, 120.
[6] T. E. Friedemann, N. F. Witt, and B. W. Neighbors, *J. Assoc. Offic. Analyt. Chem.*, 1967, **50**, 944.
[7] T. E. Friedemann and N. F. Witt, *J. Assoc. Offic. Analyt. Chem.*, 1967, **50**, 958.
[8] K. Leybold, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 51.
[9] P. Delmotte, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 46.
[10] D. A. Craven and C. W. Gehrke, *J. Chromatog.*, 1968, **37**, 414.

* See also Part I, Chapter 26.

for the determination of 2-amino-2-deoxyhexoses.[11] Resolution of eleven alditol acetates on a composite column provided a method for the analysis of neutral sugars in glycoproteins by g.l.c.[12] Maltotriose, maltose, and glucose have been analysed after reduction to the corresponding alditol and formation of the TMS derivative.[13] Urinary mono- and di-saccharides have been identified and estimated as their TMS derivatives.[13a]

A bibliography of paper chromatography and t.l.c. and a survey of applications for 1961 to 1965 has appeared .[14] Separation of the homologous series of olifosaccharides from cellobiose to cellohexaose by t.l.c. has been reported.[15] The separation by gel filtration of oligosaccharides derived from fructosans has been described.[15a]

Polygalacturonic acid has been employed as a chromatographic support for the resolution of racemic bases.[16]

Structural Methods

A method has been presented for the separation of the glycerol and erythritol glycosides of D-glucopyranose and maltose by g.l.c. which is suitable for the determination of these products in the Smith degradation of polysaccharides.[17] The use of g.l.c. in the analysis of the products from the Smith degradation of oligosaccharides has been discussed.[18] Erythritol and threitol have been separated by chromatography on an anionic resin in the molybdate form,[18a] the other products of Smith degradations being separated on an anionic resin in the sulphate form.

Spectrophotometric methods have been devised for the determination of periodate with 1,2-di-(*p*-dimethylaminophenyl)ethane-1,2-diol,[19] and iodate in the presence of periodate by masking with molybdate.[20, 21] Oxidation of starch and cellulose by periodate in aqueous DMF was retarded [22] in a similar manner to *trans*-cyclohexane-1,2-diol (R. D. Guthrie, *Chem. and Ind.*, 1960, 691).

A method for determining the distribution of substituents in partially acetylated dextran has been reported.[23] After protection of free hydroxy-

[11] M. B. Perry and A. C. Webb, *Canad. J. Biochem.*, 1968, **46**, 1163.
[12] W. F. Lehnhardt and R. J. Winzler, *J. Chromatog.*, 1968, **34**, 471.
[13] T. Cayle, F. Viebock, and J. Schiaffino, *Cereal Chem.*, 1968, **45**, 154.
[13a] T. Bhatti and J. R. Clamp, *Clin. Chim. Acta*, 1968, **22**, 563.
[14] 'Bibliography of Paper and Thin Layer Chromatography 1961–1965,' eds. K. Macek, I. M. Hais, J. Kopecký, and J. Gasparií, Elsevier, Amsterdam, 1968.
[15] S. Saif-ur-Rahmen, C. R. Krishnamurti, and W. D. Kitts, *J. Chromatog.*, 1968, **38**, 400.
[15a] H. G. Pontis, *Analyt. Biochem.*, 1968, **23**, 331.
[16] Chr. Kratchanov and M. Popova, *J. Chromatog.*, 1968, **37**, 297.
[17] C. G. S. Dutton and A. M. Unrau, *J. Chromatog.*, 1968, **36**, 283.
[18] H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1968, **63**, 553.
[18a] S. A. Barker, M. J. How, P. V. Peplow, and P. J. Somers, *Analyt. Biochem.*, 1968, **26**, 219.
[19] R. Fields and H. B. F. Dixon, *Biochem. J.*, 1968, **108**, 883.
[20] R. Belcher and A. Townshend, *Analyt. Chim. Acta*, 1968, **41**, 395.
[21] G. Nisli and A. Townshend, *Talanta*, 1968, **15**, 1377.
[22] T. P. Nevell and I. S. Shaw, *Chem. and Ind.*, 1968, 772.
[23] A. N. DeBelder and B. Norrman, *Carbohydrate Res.*, 1968, **8**, 1.

groups by reaction with methyl vinyl ether, the *O*-acetyl substituents were replaced by *O*-methyl functions. Acid hydrolysis was followed by determination of the partially methylated sugar units by g.l.c. of the corresponding alditol acetates. This technique was employed to demonstrate the site of *O*-acetylation in the O-specific side-chains of the cell wall lipopolysaccharide from *Salmonella typhimurium* 395 MS.[24]

Oxidation of the D-galactopyranosyl residues of polysaccharides to D-galactopyranosyluronic acid residues has been achieved by reaction with D-galactose oxidase followed by halogen oxidation.[25] This reaction sequence provided a method for the stabilisation of certain linkages to acid hydrolysis, as demonstrated by the isolation of 6-*O*-(α-D-galactopyranosyluronic acid)-D-mannose from oxidised guaran.[25]

Hoffman rearrangement of the amide of L-menthyl α-D-glucopyranosyluronic acid, and subsequent mild acid hydrolysis of the product, has been further reported as a method for the scission of glucuronide linkages under conditions where other types of glycosidic linkages are stable.[26] L-Ascorbic acid promoted cleavage of glycosidic bonds at a physiological pH.[27]

Reaction of polysaccharides with Procian dyes gave coloured products whose degree of substitution varied with both dye to polysaccharide ratio and with the polysaccharide structure.[28] The dyed polysaccharides gave visible bands on gel filtration or cellulose acetate electrophoresis. Heparin showed varied behaviour on Sephadex gel filtration dependent on the ionic strength of the eluant.[29] In solutions of high ionic strength heparin was retarded even on Sephadex G-100 columns. The gel filtration behaviour of heparin in comparison with *N*-desulphated heparin has been studied.[30]

N.m.r. spectroscopy has been used to investigate structural features of *Trichosporon aculeatum* mannan.[31] A comparison of the downfield shifts of H-1 proton signals on addition of borate to known mannopyranosides with the observed shifts on addition of borate to the mannan enabled structural correlations to be made. Structural studies of some oligo- and poly-saccharides by n.m.r. spectroscopy have been reported.[32]

The production of saccharinic acids in the alkaline degradation of oligosaccharides has been monitored by t.l.c. The saccharinic acids were

[24] C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Carbohydrate Res.*, 1968, **8**, 43.
[25] J. K. Rogers and N. S. Thompson, *Carbohydrate Res.*, 1968, **7**, 66.
[26] N. K. Kochetkov, O. S. Chizhov, and A. F. Sviridov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2316.
[27] J. C. Caygill, *Biochim. Biophys. Acta*, 1968, **170**, 1.
[28] W. F. D. Dudman and C. T. Bishop, *Canad. J. Chem.*, 1968, **46**, 3079.
[29] M. Skalka, *J. Chromatog.*, 1968, **33**, 456.
[30] G. B. Sumyk and C. F. Yocum, *J. Chromatog.*, 1968, **35**, 101.
[31] P. A. J. Gorin, M. Mazurek, and J. F. T. Spencer, *Canad. J. Chem.*, 1968, **46**, 2305.
[32] Yu. S. Ovodov and A. K. Dzizenko, *Doklady. Akad. Nauk S.S.S.R.*, 1968, **178**, 1338.

detected by specific spray reagents based on their distinctive periodate oxidation products.[33]

Further examples of biologically active, water-insoluble polymers prepared by the coupling of proteins to polysaccharides have been reported.[34] α-Amylase has been linked to microcrystalline cellulose *via* 3-(*p*-aminophenoxy)-2-hydroxypropyl and 2-hydroxy-3-(*p*-isothiocyanatophenoxy)propyl ethers.[35] A low degree of substitution resulted in a relatively high retention of α-amylase activity. A greater degree of heat stability and long-term storage stability was observed with the water-insoluble enzyme.

Specific Interactions of Carbohydrates with Concanavalin A and Other Proteins

Quantitative studies, using precipitin and hapten inhibition techniques, showed that bacterial α-mannans were the most reactive of a group of polysaccharides, including glycogens, amylopectins, dextrans, levans, and mannans, that formed a precipitate with the protein concanavalin A.[36] It was suggested that the combining site on concanavalin A in its reactions with α-mannans might be more extensive than hitherto believed, and might be complementary to a sequence of several α-(1 → 2)-linked D-mannopyranose units. Subsequent studies showed that, in addition to certain structural requirements of the polysaccharide, the molecular weight of the polysaccharide and the concentration of protein were important factors in determining the extent of interaction of concanavalin A with any amylopectin and glycogen.[37] A synthetic α-(1 → 6)-linked D-glucan did not form a precipitate with concanavalin A,[37a] thus supporting earlier evidence that only branched α-D-glucans precipitated with the protein. The precipitation reaction between a dextran from *Streptococcus bovis* and concanavalin A suggested that the dextran was a branched, rather than a linear, polymer. Attempts have been made to correlate the data from the precipitation reactions of other dextrans with the structures of the dextrans. A comparison of the behaviour of the concanavalin A–polysaccharide and antigen–antibody systems with regard to various parameters of turbidity has been made,[37b] and further studies of the interaction of concanavalin A with sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) and some of its derivatives have been reported.[37c]

[33] S. A. Barker, J. M. Edwards, P. J. Somers, and A. Repăs, *Carbohydrate Res.*, 1968, **6**, 341.
[34] E. M. Crook, *Biochem. J.*, 1968, **107**, 1P.
[35] S. A. Barker, P. J. Somers, and R. Epton, *Carbohydrate Res.*, 1968, **8**, 491.
[36] L. L. So and I. J. Goldstein, *J. Biol. Chem.*, 1968, **243**, 2003.
[37] E. E. Smith, Z. H. G. Smith, and I. J. Goldstein, *Biochem. J.*, 1968, **107**, 715.
[37a] I. J. Goldstein, R. D. Poretz, L. L. So, and Y. Yang, *Arch. Biochem. Biophys.*, 1968, **127**, 787.
[37b] R. D. Poretz and I. J. Goldstein, *Immunology*, 1968, **14**, 165.
[37c] I. J. Goldstein, R. N. Iyer, E. E. Smith, and L. L. So, *Biochemistry*, 1968, **7**, 482.

The ability of concanavalin A to precipitate specific polysaccharides was lost on extensive dialysis against M-acetic acid, but was restored on addition of certain bivalent cations (Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}).[38] High concentrations ($>0{\cdot}05$M) of such cations, however, were inhibitory to the concanavalin–polysaccharide interaction. Other workers showed that sites for binding methyl α-D-glucopyranoside on the concanavalin molecule existed only when a transition metal and Ca^{2+} ions were bound.[39] From the results of equilibrium dialysis studies using concanavalin A with methyl α-D-mannopyranoside and methyl α-D-glucopyranoside, So and Goldstein concluded[40] that concanavalin A was bivalent. Similar conclusions were reached by Kalb and Lustig.[41] Other studies of factors affecting the interaction of glycogen with concanavalin A showed that the complex dissociated in the range 30–50°, that (ethylene dinitrilo)tetra-acetic acid slightly inhibited complex formation, and that formamide and various urea derivatives acted as competitive inhibitors.[42] Complex formation with concanavalin A did not protect glycogen from glycogenolysis with α- or β-amylase.

Precipitin-like reactions have been demonstrated between gelatin and a wide range of neutral and acidic (lipo)polysaccharides in aqueous solution.[43]

[38] B. B. L. Agrawal and I. J. Goldstein, *Canad. J. Biochem.*, 1968, **46**, 1147.
[39] A. J. Kalb and A. Levitski, *Biochem. J.*, 1968, **109**, 669; J. Yariv, A. J. Kalb, and A. Levitski, *Biochem. Biophys. Acta*, 1968, **168**, 303.
[40] L. L. So and I. J. Goldstein, *Biochem. Biophys. Acta*, 1968, **165**, 398.
[41] A. J. Kalb and A. Lustig, *Biochem. Biophys. Acta*, 1968, **168**, 336.
[42] R. J. Doyle, E. P. Pittz, and E. E. Woodside, *Carbohydrate Res.*, 1968, **8**, 89.
[43] E. E. Woodside, G. F. Trott, R. J. Doyle, and C. W. Fishel, *Carbohydrate Res.*, 1968, **6**, 449.

3
Plant Polysaccharides

The pectic substances of *Zosteraceae* have been studied, and the polysaccharide composition of several plants were found to be nearly identical.[44] A homogeneous pectic polysaccharide, designated zosterine, contained D-galacturonic acid, D-galactose, D-xylose, L-arabinose, D-apiose, and a mono-*O*-methyl-D-xylose. The preparation contained 38–40% uronic anhydride, 2·3–2·45% nitrogen and 0·97–0·80% methoxy-groups. The pectic substances from *Dianthus caryophyllus* L. (carnation roots) contained D-galacturonic acid, D-galactose, and L-arabinose (55 : 24 : 16).[45] Fractionation gave a distribution of components containing varying proportions of acidic groups. 2-*O*-(D-Galactosyluronic acid)-(1 → 2)-rhamnose, *O*-(D-galactosyluronic acid)-D-galactose, and *O*-(-D-galactosyluronic acid)-L-arabinose were isolated from partial acid hydrolysates.

Three pectin fractions from lemon peel have been investigated.[46] Pectin A on enzymic hydrolysis gave *inter alia* 3-*O*-xylopyranosylgalacturonic acid, and tentatively 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-L-rhamnose and galactopyranosyluronic acid-(1 → 2)-rhamnopyranosyl-(1 → 4)-galacturonic acid. Pectin B, $[\alpha]_D + 221°$, containing 76% uronic acid anhydride and 10·2% *O*-methyl groups, still contained a small amount of a neutral polysaccharide contaminant. Partial acid hydrolysis gave *inter alia* 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose and higher oligomers, 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid and the polymer homologous trimer, together with 6-*O*-(glucopyranosyluronic acid)-galactose, 4-*O*-(glucopyranosyluronic acid)-fucose and 3-*O*-xylopyranosylgalacturonic acid. It was suggested that rhamnose units interrupted blocks of (1 → 4)-linked galacturonic acid units in the main chain. Acetolysis of Pectin C, obtained by DEAE cellulose chromatography, gave *inter alia* galactopyranosyluronic acid-(1 → 2)-rhamnopyranosyl-(1 → 2)-rhamnose.

The distribution of free carboxyl groups in a pectin molecule after treatment with pectin esterase has been determined.[47] The stability constant of the calcium salt of partially de-esterified pectin was much higher than that

[44] R. G. Ovodova, V. E. Vaskovsky, and Yu. S. Ovodov, *Carbohydrate Res.*, 1968, **6**, 328.
[45] M. J. Foglietti and F. Percheron, *Carbohydrate Res.*, 1968, **7**, 146.
[46] G. O. Aspinall, J. W. T. Craig, and J. L. Whyte, *Carbohydrate Res.*, 1968, **7**, 442.
[47] R. Kuhn, I. Furda, and Z. Kopee, *Coll. Czech. Chem. Comm.*, 1968, **33**, 264.

obtained with alkaline de-esterification, indicating a blockwise arrangement of free carboxyl groups in the enzyme-treated pectin.

Further structural features of the pectic substances from lucerne (*Medicago sativa*) have been elucidated.[48] Acidic oligosaccharides have been obtained from partial hydrolysis of leaf and stem pectic acids (1).

Oligosaccharide	*Pectin from*			
	Stem		*Leaf*	
	a	*b*	*a*	*b*
α-D-Gal*p*A-(1→2)-L-Rha	+	+	+	+*
β-D-G*p*A-(1→4)-L-Fuc	+	−	+	−
β-D-G*p*A-(1→6)-D-Gal	+	−	+	−
Gal*p*A-(1→2)-Rha*p*-(1→4)-Gal*p*A-(1→2)-Rha	+	+	+	+*
Gal*p*A-(1→4)-Gal*p*A-(1→2)-Rha	−	+	+	−
α-D-Gal*p*A-(1→4)-D-Gal*p*A	+	−	+	−
α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-D-Gal*p*A	+	−	+	−
Gal*p*A-(1→2)-Rha*p*-(1→2)-Rha*p*	−	+	−	+*

a = Partial acid hydrolysis; *b* = acetolysis; * detection by chromatography only.

(1)

Xylose was identified as an integral part of the lucerne pectic acid by the isolation from pectinase digests of 3-*O*-xylopyranosylgalacturonic acid. Characterisation of 3-*O*-arabinofuranosylgalacturonic acid provided the first conclusive evidence of arabinose as a constituent of pectic acid. Fractionation of the water-soluble polysaccharides by DEAE-Sephadex chromatography gave neutral and acidic arabinogalactans of highly branched structures, and a series of pectinic acids of varying ester content.[48a] No branching through arabinose was found although certain structural features were established for the arabinogalactan (2) and the acidic polysaccharide (3).

Ara*f* 1→...; ...→2-Ara*f* 1→...; ...→5-Ara*f* 1→...;
Ara*p*-(1→3)-Ara*f* 1→...; Gal*p* 1→...; ...→3 Gal*p* 1→...; ...→4 Gal*p* 1→...;
...→6 Gal*p* 1→...; ...→3 Gal*p* 1→...
6
↑
⋮ (2)

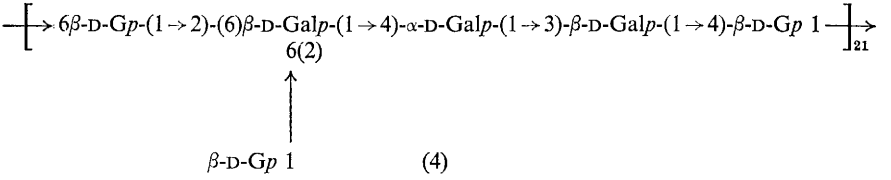
...→4 Gal*p* A(1→2)-Rha*p* 1→...; D-G*p*A1→...
4
↑
⋮ (3)

[48] G. O. Aspinall, B. Gestetner, J. A. Molley, and M. Uddin, *J. Chem. Soc.* (*C*), 1968, 2554.

[48a] G. O. Aspinall and J. A. Molley, *J. Chem. Soc.* (*C*), 1968, 2994.

A particulate hemicellulose from *Phaseolus aureus* shoots contained D-glucose, D-galactose, L-arabinose, and D-mannose.[49] Partial acid hydrolysis gave cellobiose, 3-*O*-β-L-arabinosyl-L-arabinose, di- and tri-galacturonic acids, D-galactosyluronic acid-L-rhamnose, D-glucosyluronic acid-D-galactose, and D-glucosyluronic acid-D-mannose. These findings suggested the presence *inter alia* of a typical polygalacturonic acid and an arabinogalactan complex.

A β-D-(1 → 6)-glucanase from *Trichoderma viride* fragmented kefiran, the water-soluble D-galacto-D-glucan (1 : 1)[50] of the kefir grain, into equimolar proportions of glucose and a pentasaccharide, kefirose.[50a] Methylation, periodate oxidation, and partial acid hydrolyses of both kefiran and kefirose enabled a structure of kefiran to be postulated (4). The molecular



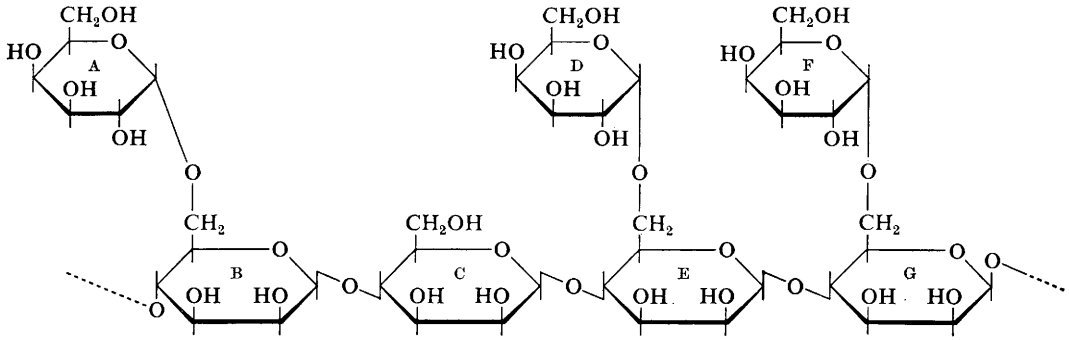
(4)

weight of kefiran was 20,600 corresponding to an average degree of polymerisation ($\overline{DP}$) of 127 by the hypoiodite method.

Moghat, from the sun-dried roots of *Clossostemon bruguieri*, has been shown to contain two polysaccharides accounting for 70% of the mucilage.[51] Polysaccharide A, $[\alpha]_D$ −200° and $\overline{DP}$ 31, was obtained as an insoluble copper complex and consisted of (1 → 4)-linked D-glucopyranosyl residues (77%) and L-rhamnose (20%). The soluble copper complex, component B, was a glycogen-like polymer, $[\alpha]_D$ −220° and $\overline{DP}$ 18, containing D-glucose (89%) and L-arabinose (9%).

Structural studies of galactomannans from numerous sources have been reported. A galactomannan from the seeds of *Lotus pedunculatus* contained[52] a linear main chain of (1 → 4)-linked β-D-mannopyranosyl units (51%) with side chains of α-D-galactopyranosyl units (49%) attached to C-6 of *c.a.* six out of every seven D-mannopyranosyl units. An essentially similar galactomannan has been obtained from *Medicago lupulina*[52a] and a partial structure (5) proposed on the basis of periodate oxidation, methylation, enzymic and acid hydrolyses. In addition to 4-*O*-β-D-mannopyranosyl-D-mannose and 6-*O*-α-D-galactopyranosyl-D-mannose partial hydrolysis gave trisaccharides ABC and CDE, together with tetrasaccharides ABCE, CDEG, and CEFG [see (5)]. The galactomannan fom the seeds of *Sesbania*

[49] R. W. Bailey, S. Haq, and W. Z. Hassid, *Phytochem.*, 1967, **6**, 293.
[50] J. W. M. LaRiviere, P. Kooiman, and K. Schmidt, *Arch. Mikrobiol.*, 1967, **59**, 269.
[50a] P. Kooiman, *Carbohydrate Res.*, 1968, **7**, 200.
[51] El. S. Amin and O. M. Awad, *Carbohydrate Res.*, 1968, **7**, 12.
[52] E. L. Richards, R. J. Beveridge, and M. R. Grimmett, *Austral. J. Chem.*, 1967, **21**, 2107.
[52a] R. Somme, *Acta Chem. Scand.*, 1968, **22**, 870.



(5)

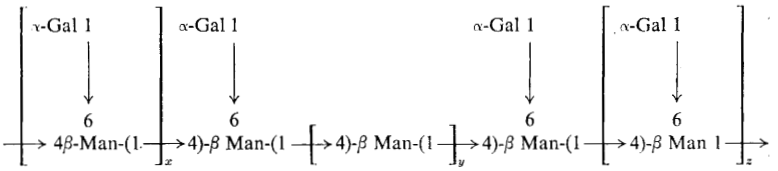
(6) → 4D-Man*p*-(1→4)-D-Man*p* 1 →$_n$
6
↑
D-Gal*p* 1

(7) → 4D-Man*p*-(1→6)-D-Man*p* 1 →$_n$
4
↑
D-Gal*p* 1

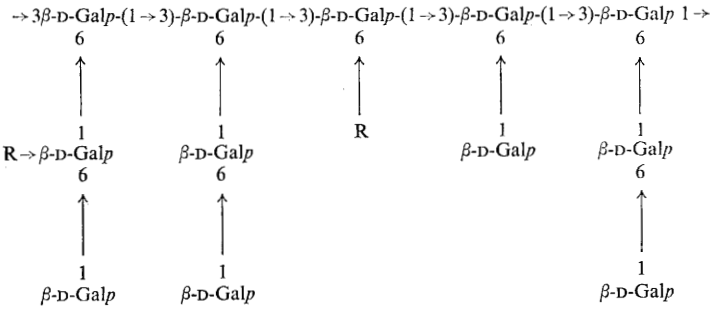
(8) → 4D-Man*p* 1 →$_n$
6
↑
D-Gal*p*-(1→4)-D-Man*p* 1

(9) → 6D-Man*p* 1 →$_n$
4
↑
D-Gal*p*-(1→4)-D-Man*p* 1

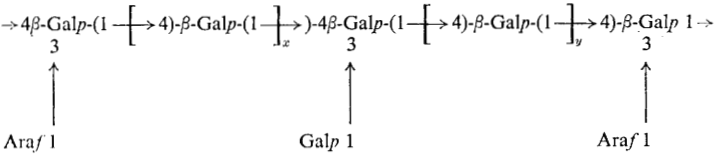
grandiflora pers was similar in structure although the data obtained did not allow the differentiation of several possible repeating units (6–9).[53] A highly branched galactomannan was obtained from the seeds of *Ipomoea muricata* with a D-galactose : D-mannose ratio of 1 : 1·8.[53a] Methylation and periodate oxidation allowed the postulation of a general structure (10). The distribution of galactose and mannose in the cell-wall polysaccharides of red clover (*Trifolium pratense*) leaves and stems has been determined.[54]



(10) $x+z=3$; $y=4$



(11) R = L-Araf 1→ ; or β-L-Araf-(1→3)-L-Araf 1→



(12) $x+y=4$

The main structural features of arabinogalactan A from Japanese larch (*Larix leptolepis*) have been determined (11) by hydrolysis, methylation, and Smith degradation studies.[55] Oxidation of a partially methylated sample of the arabinogalactan with chromium trioxide–acetic acid followed by partial acid hydrolysis allowed the isolation of *inter alia* partially

[53] H. C. Srivastava, P. P. Singh, and P. V. Subba Rao, *Carbohydrate Res.*, 1968, **6**, 361.
[53a] S. N. Khanna and P. C. Gupta, *Phytochem.*, 1967, **6**, 605.
[54] B. D. E. Gaillard and R. W. Bailey, *Phytochem.*, 1968, **7**, 2037.
[55] G. O. Aspinall, R. M. Fairweather, and T. M. Wood, *J. Chem. Soc.* (*C*), 1968, 2174.

methylated aldobiuronic acids. This demonstrated that 6-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl- and 6-*O*-β-D-galactopyranosyl-(3-*O*-glycosyl)-β-D-galactopyranosyl- were structural features of the original polysaccharide. Examination of the gum from the tree *Lannea coromandelica* showed that it consisted of a neutral polysaccharide composed of D-galactose and L-arabinose in the ratio 4 : 1.[55a] Periodate oxidation and methylation indicated that the structure could be represented by (12).

The glucomannan from the roots of *Eremurus fuscus* was hydrolysed to glucose and mannose by an endopolyglycosidase from *Aspergillus oryzae*.[56]

A number of reports have appeared concerning various aspects of the chemistry of the gums from several *Acacia* species. An analytical study of different forms of gum from *A. senegal* Willd has been made using 12 bulk samples from three districts of the Sudan and 13 different single nodules of 'natural exudate'.[57] The samples showed the same range of variation of analytical parameters as with previous samples of other *Acacia* species. Samples of non-nodular gum and of gum from a wood-boring beetle-infected tree failed to show significantly different analyses. 'Hennawi' gum from the main stem of trees showed fewer free uronic acid residues and a lower rhamnose content. A comparative structural study using methylation and Smith degradation, was performed on the normal tapped gum, 'Hennawi' gum, and the gum from the infected tree.[57a] The gum from the infected tree and that from the normal exudate were virtually identical, whilst the 'Hennawi' gum differed mainly in the peripheral end group positions of L-rhamnose and D-glucuronic acid. A generalisation has been suggested relating the optical rotation and methoxy-content of *Acacia* gums with the aldobiuronic acids obtainable by partial acid hydrolysis.[58]

The gum polysaccharide from *A. nubica* benth contained L-rhamnose, D-glucuronic acid, L-arabinose, and D-galactose (1 : 7 : 59 : 33) and had a methoxy-content of 0·05–0·1%.[59] Autohydrolysis, partial hydrolysis, methylation, and repetitive Smith degradations indicated a highly branched D-galactan framework with D-glucuronic acid residues and arabinose side-chains, some of which were six units long, as the main structural features. The structure was thus markedly different from that of *A. senegal* gum but similar in many respects to that of *A. arabica* gum polysaccharide. Some structural data have been obtained from the *A. laeta* var. hashab gum.[60] Methylation of the autohydrolysed gum and eight Smith-degraded gums allowed a possible structural fragment to be proposed although unequivocal proof of a main chain of β-(1 → 3)-linked D-galactose residues was

[55a] R. Ramachandran and B. C. Joshi, *Phytochem.*, 1968, **7**, 2057.
[56] A. A. Kuznetsov and B. N. Stepanenko, *Biokhimiya*, 1967, **32**, 368.
[57] D. M. W. Anderson, I. C. M. Dea, K. A. Karamalla, and J. F. Smith, *Carbohydrate Res.*, 1968, **6**, 97.
[57a] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1968, **6**, 104.
[58] D. M. W. Anderson and G. M. Cree, *Carbohydrate Res.*, 1968, **6**, 215.
[59] D. M. W. Anderson and G. M. Cree, *Carbohydrate Res.*, 1968, **6**, 385.
[60] D. M. W. Anderson, I. C. M. Dea, and R. N. Smith, *Carbohydrate Res.*, 1968, **7**, 320.

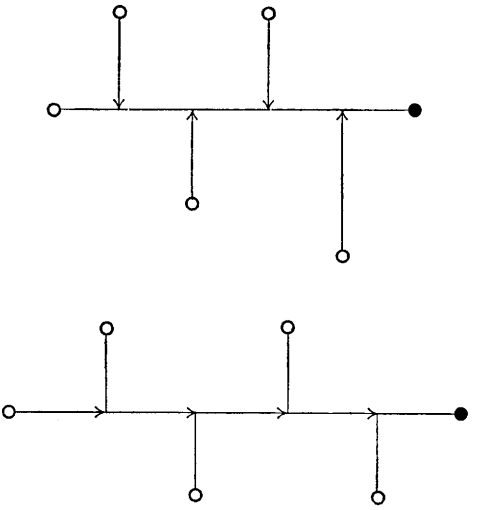
not obtained (13). Several possible arrangements for the galactan framework of this gum polysaccharide could be postulated (14) but not distinguished.

$$\rightarrow 3\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\ 1\rightarrow$$

$$\begin{array}{c} 3 \\ \uparrow \\ \mid \\ \mathrm{R} \end{array}$$

R = L-Ara$p$ 1→; L-Ara$f$ 1→; →6D-Gal$p$ 1→; or D-Gal$p$ 1→

(13)



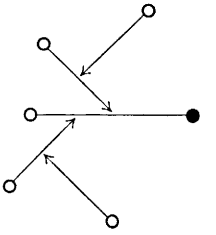
(14)

— β-(1→3)-D-Gal$p$ chains

→ β-(1→6)-linkages at branch points

● reducing terminus

o non-reducing termini



Analytical and structural data on three fractions of the gum from *A. drepanolobium* indicated that they were structurally similar.[61] Fraction

[61] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1968, **8**, 440.

A from the *A. drepanolobium* Harms ex Sjöstedt gum contained D-galactose (38%), L-arabinose (52%), L-rhamnose (1%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (2%).[61a] Methylation, periodate oxidation, and successive Smith degradations indicated that arabinose side-chains, some of which were considerably longer than in other *Acacia* gums, were attached to a compact branched galactan framework. Non-reducing termini were L-rhamnose and 4-*O*-methyl-D-glucuronic acid groups. The information obtained allowed many structural fragments to be recognised, (15) and (16).[61b] To the galactan backbone [blocks of β-(1 → 3)-linkages

L-Ara*f* 1 → ...; L-Ara*p* 1 → ...

α-D-G*p*A-(1 → 4)-D-Gal*p* 1 →; β-D-G*p*A-(1 → 6)-D-Gal*p* 1 →;
α-D-G*p*A-(1 → 4)-D-Gal*p* 1 →; β-D-G*p*A-(1 → 6)-D-Gal*p* 1 →;
3 3
↑
|

or the 4-*O*-Me-D-G*p*A analogues

β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 6)-D-Gal*p* 1 →
β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*p* 1 →

(15)

Fragments of autohydrolysed *A. drepanolobium* gum.

Possible structural feature of autohydrolysed *A. drepanolobium* gum*

→ 3β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 3)-D-Gal*p* 1 →
3
↑
|
R

where R = L-Ara*f* 1 → ; L-Ara*p* 1 →; D-Gal*p* 1 →; → 4D-Gal*p* 1 →
or → 6D-Gal*p* 1 →

(16)

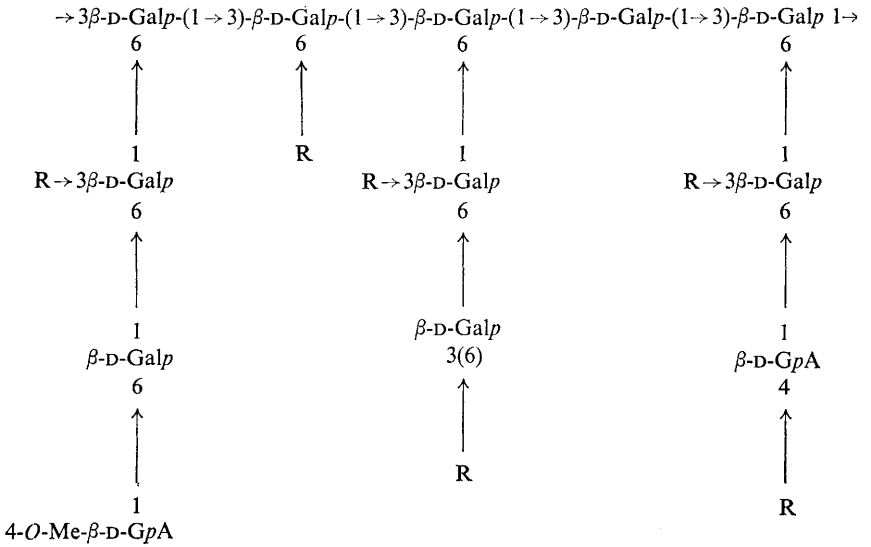
* Intact polysaccharide also contains → β-L-Ara*f*-(1 → 3)-L-Ara*f*1 → and → β-L-Ara*p*-(1 → 3)-L-Ara*f*1 → fragments.

interspaced with β-(1 → 6)-linkages] were attached some single arabinose units, some short chains (< 5 units), and some long chains [> 8 units, predominantly (1 → 2)-linked]. This polysaccharide was more expanded than that from *A. arabica*, consistent with its intrinsic viscosity and gel permeation behaviour.

[61a] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1968, **8**, 448.
[61b] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1968, **7**, 109.

The gum from a single nodule of *A. seyal* contained D-galactose (38%), L-arabinose (45%), L-rhamnose (4%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (6%).[62] Structural studies indicated a strong similarity between this gum and that from *A. arabica*. A highly branched galactan framework was proposed with uronic acid residues and arabinose side-chains attached, whose length was less than those of *A. arabica* and much less than those of *A. drepanolobium* gum.

The exudate gum from *A. mearnsii* was composed of galactose, arabinose, rhamnose, and glucuronic acid and its 4-*O*-methyl ether (5·6 : 4·9 : 1·0 : 1·6).[63] Combinations of methylation and Smith degradations allowed a possible structure to be written (17). No major differences were observed between



R = L-Ara*f* 1→; L-Ara*f*-(1→3)-L-Ara*f* 1→; L-Arab*f*-(1→5)-L-Ara*f* 1→; L-Rha*p* 1→ or β-L-Ara*p*-(1→3)-L-Ara*f* 1→

(17)

gum from South African or Jamaican trees of the same species. It was concluded that samples of gum from the same botanical species showed no apparent differences in the nature of sugar residues or in the linkages, although there may be small differences in the relative proportions of these residues.

[62] D. M. W. Anderson, I. C. M. Dea, and Sir Edmund Hirst, *Carbohydrate Res.*, 1968, **8**, 460.

[63] G. O. Aspinall, J. L. Carlyle, and R. Young, *Carbohydrate Res.*, 1968, **7**, 421.

2-*O*-β-L-Arabinofuranosyl-L-arabinose and 3-*O*-β-L-arabinopyranosyl-L-arabinose have been characterised in the autohydrolysate of *A. niletica* gum.[64]

Further studies of the polysaccharide components of *Araucaria bidwilli* gum have been reported.[65] An arabinogalactan (4 : 1), $[\alpha]_D$ +30°, and a highly branched acidic polysaccharide, $[\alpha]_D$ −9·5°, have been isolated. Some structural features have been elucidated for each polysaccharide.

The average analyses for the gum from ten nodules indicated that sapote gum consisted of D-xylose, L-arabinose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid (2·2 : 1·0 : 0·42 : 0·58).[66] Two trisaccharides, (18) and (19), were characterised in a partial acid hydrolysate of the gum.

(18) 4-*O*-Me-α-D-G*p*A-(1→2)-β-D-Xyl*p*-(1→4)-D-Xyl
(19) α-D-G*p*A-(1→2)-β-D-Xyl*p*-(1→4)-D-Xyl

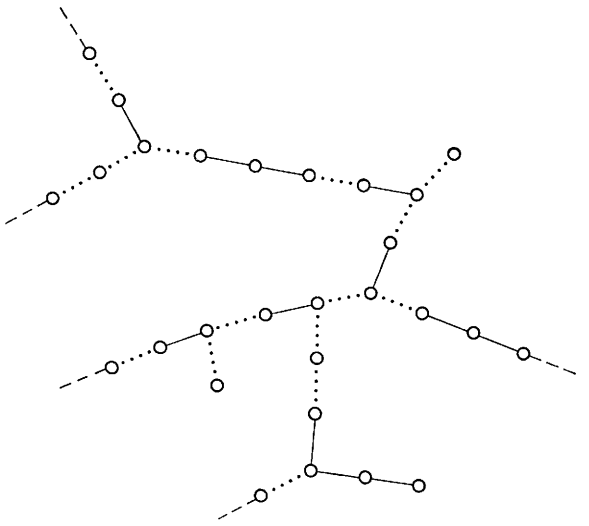
Some structural features of *Citrus limonia* gum (lemon gum) have been elucidated.[67] This gum was composed of D-galactose (36%), L-arabinose (33%), L-rhamnose (5%), D-glucuronic acid (7%), and 4-*O*-methyl-D-glucuronic acid (16%). Linkage analysis, methylation, and Smith degradation of native gum and of graded acid-hydrolysed gum enabled a probable structure to be described (20).

The structural features of polysaccharides from apricot tree gum have been studied with respect to infection of the tree by pathogens and application of 2-methyl-4-chlorophenoxyacetic acid.[67a]

Birch sulphate pulp, eucalypt pulp, pine sulphate, and eucalypt neutral sulphite pulp prepared under alkaline conditions all contained a methylated uronic acid.[68] This was identified as 4-*O*-methyl-L-iduronic acid formed by epimerisation at C-5 of 4-*O*-methyl-D-glucuronic acid from xylan during the alkaline cooking process. The factors involved in the extraction of a pentosan from hardwood with sodium hydroxide have been investigated.[69] Treatment of an arabinoxylan from wheat-flour with an α-L-arabinofuranosidase gave arabinose and a xylan.[70] Treatment of a wheat-flour glycoprotein with pronase resulted in the formation of two polysaccharides of different sugar composition.[70]

In response to wounding or infection the saguaro cactus formed a hard, ligniferous callus tissue containing essentially water-insoluble cellulose and

[64] R. C. Chalk, J. F. Stoddart, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1968, **46**, 2311.
[65] G. O. Aspinall and J. P. McKenna, *Carbohydrate Res.*, 1968, **7**, 244.
[66] R. D. Lambert, E. E. Dickey, and N. S. Thompson, *Carbohydrate Res.*, 1968, **6**, 43.
[67] J. F. Stoddart and J. K. N. Jones, *Carbohydrate Res.*, 1968, **8**, 29.
[67a] J. Rosík, J. Kubala, M. Stanová, and P. Laćok, *Collect. Czech. Chem. Commun.*, 1968, **33**, 1943.
[68] E. R. Nelson, P. F. Nelson, and O. Samuelson, *Acta Chem. Scand.*, 1968, **22**, 691.
[69] R. Kobayashi, Y. Sakai, and T. Kobayashi, *J. Ferment. Technol.*, 1968, **46**, 753.
[70] H. Nenkom, L. Providoli, H. Gremli, and P. A. Hui, *Cereal Chem.*, 1967, **44**, 238.



O = D-Gal*p* —— = β-(1→3) ···· = β-(1→6)

(20)

Hexuronic acid and rhamnose substituents attached to the galactan framework.

a xylan.[71] Healthy cortical tissue contained a relatively water-soluble mixture of polysaccharides.

Polysaccharides from the cortical and heart regions of the lenkoran hollyhock contained arabinose, xylose, rhamnose, fructose, glucose, galactose, 2-amino-2-deoxy-glucose, and galacturonic acid.[72]

Fifteen major and five minor low molecular weight components have been isolated from potato (*Solanum tuberosum*).[73] The trisaccharide fraction of some plants belonging to the *Amaryllidaceae* has been examined and three fructosyl-sucroses characterised.[74]

A galactan of probable molecular weight at least 4600 has been synthesised from UDP-D-(^{14}C)-galactose by a particulate cell-free enzyme system from *Phaseolus aureus* shoots.[75] Seeds of *Dolichos biflorus* contain a glycoprotein (M_w 130,000) with mannose (0·2%) and galactose (0·5%) together with traces of rhamnose and fucose, which reacts against the streptococcal agglutinin of the serotype C.[76]

A study of the oxidation of amylose with acidified solutions of sodium chlorite has led to the suggestion that the small quantities of glucuronic

[71] C. Steelink, E. Riser, and M. J. Onore, *Phytochem.*, 1968, **7**, 1673.
[72] I. S. Kozhina and N. A. Trukhaleva, *Doklady. Akad. Nauk S.S.S.R.*, 1967, **177**, 458.
[73] B. Urbas, *Canad. J. Chem.*, 1968, **46**, 49.
[74] H. Hammer, *Acta Chem. Scand.*, 1968, **22**, 197.
[75] J. M. McNab, C. L. Villemez, and P. Albersheim, *Biochem. J.*, 1968, **106**, 355.
[76] O. Kuhnemund, W. Kohler, and O. Prokop, *Z. Physiol. Chem.*, 1968, **349**, 1434.

acid, often found in acid hydrolysates of wood holocelluloses, could arise in an artifactual manner through oxidation of the primary hydroxy-group in the glucose residues of cellulose and glucomannans.[77]

A method has been described for the investigation of the fine structure of amylopectin.[78] After fragmentation into unit chains with pullulanase, the oligosaccharides were fractionated by gel filtration and their chain length determined by reaction with iodine. Conclusions could be drawn as to the most probable lengths of the A and B chains of amylopectins. A correlation between theoretically derived rates and measured rates of attack of various α-amylases on amylopectin, amylopectin β-limit dextrin, glycogen, and glycogen β-limit dextrin demonstrated that soya bean α-amylase was better able to penetrate the polysaccharide matrices.[79] Barley limit dextrinase has been shown to have similar minimum structural requirements for action to the pullulanase from *Aerobacter aerogenes*.[80] A branching enzyme from sweet corn (amylopectin-branching glycosyl transferase), which can be separated from the normal plant branching enzyme (Q enzyme) by gel filtration, was shown to introduce additional inter-chain linkages into amylopectin thereby producing a glycogen-type polysaccharide.[80a] Erythritol and sucrose have been shown to inhibit the degradation of both maltose and glycogen by γ-amylase.[81] αα-Trehalose and methyl-α-D-glucopyranoside inhibited the degradation of glycogen but not of maltose.

2-Deoxy-D-*arabino*-hexose was shown to be incorporated into glycogen in an analogous manner to the glucosyl transfer from UDP-glucose into glycogen.[82] 4-*O*-α-(2-Deoxy-D-*arabino*-hexopyranosyl)-2-deoxy-D-*arabino*-hexose, but not 4-*O*-α-D-glucopyranosyl-2-deoxy-D-*arabino*-hexose, was reported in enzyme digets of the 'glycogen'.

The fractionation, specificity, and inhibition of α-L-arabinofuranosidases have been described.[83]

[77] C. M. Stewart and J. A. Smelstorius, *Chem. and Ind.*, 1968, 618.
[78] E. Y. C. Lee, C. Mercier, and W. J. Whelan, *Arch. Biochem. Biophys.*, 1968, **125**, 102.
[79] R. Geddes, *Carbohydrate Res.*, 1968, **7**, 493.
[80] D. J. Manners and K. L. Rowe, *Biochem. J.*, 1968, **110**, 35P.
[80a] D. J. Manners, J. J. M. Rowe, and K. L. Rowe, *Carbohydrate Res.*, 1968, **8**, 72.
[81] E. L. Rosenfeld and D. M. Belenki, *Bull. Chem. Soc. Biol.*, 1968, **50**, 1305.
[82] P. Biely, V. Farkaš, and Š. Bauer, *Biochim. Biophys. Acta*, 1968, **158**, 487.
[83] D. J. Manners and D. C. Taylor, *Carbohydrate Res.*, 1968, **7**, 497; H. Gremli and H. Neukom, *Carbohydrate Res.*, 1968, **8**, 110; J. Conchie, A. L. Gelman, and G. A. Levy, *Biochem. J.*, 1968, **106**, 135.

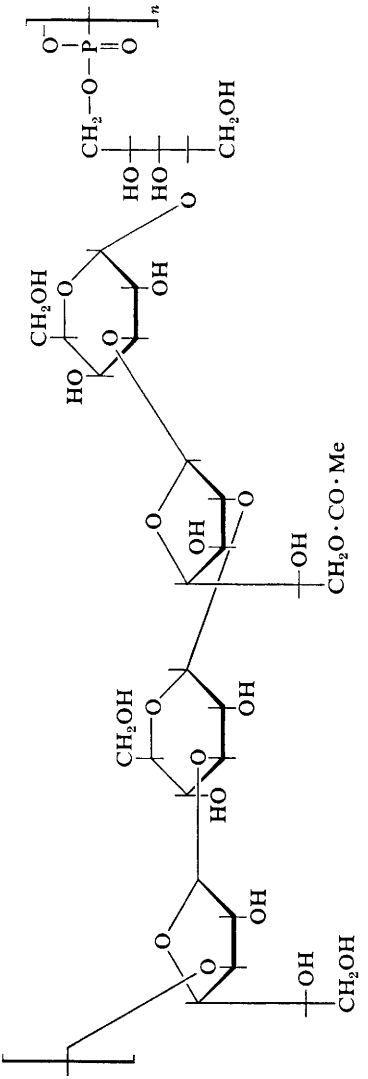
4
Bacterial Polysaccharides

Further structural features of the type specific polysaccharide (S XXXIV) from *Diplococcus pneumoniae* Type 34 (U.S. Type 41) have been elucidated by two groups. Partial acid hydrolysis gave the disaccharide, 2-*O*-(α-D-glucopyranosyl)-D-galactose, whilst 2-*O*-(α-D-glucopyranosyl)-L-arabinose was obtained after deacetylation, periodate oxidation, borohydride reduction, and partial acid hydrolysis.[84] Type S XXXIV itself gave a mixture of these two products after Smith degradation. The phosphodiester linkages were shown to join the hydroxy-group at C-1 or C-5 of ribitol and the hydroxy-group at C-3 of a D-galactofuranose group in the next repeating unit.[84a] Deacetylation with ammonia caused partial loss of immunological activity. The homologous antigen–antibody precipitin reaction was strongly inhibited by synthetic methyl 6-*O*-acetyl-β-D-galactofuranoside, whereas methyl β-D-galactofuranoside showed no such inhibitory powers, strongly suggesting that the *O*-acetyl-group in S XXXIV was located on the C-6 position of an interior D-galactofuranose residue.[84b] These observations allowed the formulation of a repeating unit (21) for S XXXIV.[84]

The Forssman antigen has been purified from a *rough* variant of *Diplococcus pneumoniae* Type 1.[85] The polysaccharide, $[\alpha]_D$ +24°, contained 2-amino-2-deoxy-galactose, glucose, and ribitol (probably as ribitol phosphate) and appeared homogeneous by ultracentrifugation, immunoelectrophoresis, and gel-diffusion. Small amounts of fatty acids (6·6%) and protein (4%) were also detected in the preparation.

The specific polysaccharide (S VII) from *Diplococcus pneumoniae* Type VII was composed of D-galactose, D-glucose, L-rhamnose, and 2-acetamido-2-deoxy D-glucose and 2-acetamido-2-deoxy-D-galactose (4 : 2 : 3 : 2 : 2).[85a] Inhibition studies on the cross-reaction of tamarind seed mucilage with antipneumococcal Type VII serum warranted the prediction that β-linked D-galactopyranosyl non-reducing termini would be found in S VIII.

[84] J. R. Dixon, W. K. Roberts, G. T. Mills, J. G. Buchanan, and J. Baddiley, *Carbohydrate Res.*, 1968, **8**, 262.
[84a] G. J. F. Chittenden, W. K. Roberts, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, 1968, **109**, 597.
[84b] N. Roy and C. P. J. Glandemans, *Carbohydrate Res.*, 1968, **8**, 214.
[85] M. Fujiwara, *Jap. J. Exptl. Med.*, 1967, **37**, 581.
[85a] J. M. Tyler and M. Heidelberger, *Biochemistry*, 1968, **7**, 1384.



(21)

Further studies have been reported on the structure of *D. pneumoniae* C-substance.[86] Extraction of the non-capsulated *D. pneumoniae* Type I with trichloracetic acid followed by fractionation on DEAE-cellulose gave a material, $[\alpha]_D$ +74°, containing phosphate, 2-acetamido-2-deoxy-D-galactose, D-glucose, a diacetamido-trideoxyhexose, ribitol, and choline (2 : 1 : 1 : 1 : 1 : 1). Acid hydrolysis gave 2-amino-2-deoxy-D-galactose hydrochloride, 2-amino-2-deoxy-D-galactose-6-phosphate, anhydroribitol, and D-glucose whilst partial acid hydrolysis gave *inter alia* a product provisionally identified as 6′-*O*-phosphoryl-[*O*-β-2-amino-2-deoxy-D-galactosyl-(1 → 6)-D-glucose]. Both choline phosphate and ribitol phosphate were units in the polymer. C-Substances from seven other strains of *D. pneumoniae* possessed a structure common to that found in the type described. Capsulated mutants of *D. pneumoniae* producing a capsule of soluble polysaccharide related immunologically to the C- or cell-wall-polysaccharide have been isolated from several non-capsulated varients of this organism.[87] The binding of labelled oligosaccharides derived from *D. pneumoniae* Type VIII specific polysaccharide with anti-S VIII antibodies has been studied.[87a]

The dextran from *Leuconostoc mesenteroides* NRRL B-512 and two fractions, dextran 10 and dextran 80 produced by graded acid hydrolysis, have been investigated with particular reference to the nature of the side-chains.[88] Methylation studies showed that all the main chain residues were α-(1 → 6)-linked and that all branch points started from the C-3-position in the glucose residues. Carboxydextrans, produced by catalytic oxidation, gave 6-*O*-(α-D-glucopyranosyluronic acid)-D-glucose as the predominant aldobiuronic acid indicating that in the parent dextran most of the side-chains were larger than one glucose unit in length. The presence of some α-(1 → 3)-linked aldobiuronic acid was demonstrated. The major aldotriuronic acid component isolated from oxidised dextran 10 was identified as *O*-(α-D-glucopyranosyluronic acid)-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose. The dextran from *L. mesenteroides* strain C has been investigated by methylation and fragmentation analysis.[88a] The distribution of *O*-methyl groups in partially methylated dextran (using dimethyl sulphate in alkaline solution) was determined by hydrolysis and g.l.c.[89] The ratio of relative rate constants for methylation of hydroxy-groups at C-2, C-3, and C-4 was observed as 8·0 : 1·0 : 3·5, the reactivity of the C-3-hydroxy-group being enhanced when those at C-2 and C-4 were methylated whereas the reactivities of the C-2 and C-4-hydroxy-groups are unaffected by substitution at C-3.

[86] D. E. Brundish and J. Baddiley, *Biochem. J.*, 1968, **110**, 573.
[87] D. L. Bornstein, G. Schiffman, H. P. Bernheimer, and R. Austrian, *J. Exptl. Med.*, 1968, **128**, 1385.
[87a] A. M. Pappenheimer jun., W. P. Reed, and R. Brown, *J. Immunol.*, 1968, **100**, 1237.
[88] B. Lindberg and S. Svensson, *Acta Chem. Scand.*, 1968, **22**, 1907.
[88a] B. A. Lewis, M. J. St. Cyr, and F. Smith, *J. Org. Chem.*, 1968, **33**, 3136, 3139.
[89] B. Norrman, *Acta Chem. Scand.*, 1968, **22**, 1381.

A cell-bound exodextranase has been isolated from cultures of *Bacillus subtilis* and *B. megathesium*.[89a]

Three types of β-D-glucan have been extracted from the cell-walls of *Aureobasidium* (*Pullularia*) *pullulans*.[90] One polysaccharide, extracted with dilute alkali, consisted of an essentially linear β-(1 → 3)-linked glucan with occasional single D-glucose residues substituted at the C-6-positions. The cell-wall material insoluble in alkali consisted of two polysaccharides, the first mainly a linear β-(1 → 3)-linked D-glucan and highly crystalline, and the second containing (1 → 3)- and (1 → 6)-linked D-glucose residues. In addition the cell-wall contained a heteropolysaccharide consisting of D-mannose, D-galactose, D-glucose, and D-glucuronic acid.[90a] Methylation and periodate oxidation data indicated a chain of (1 → 6)-linked D-mannopyranose units, most of which were substituted in the C-3-position with either β-D-glucopyranosyl units, a short chain of (1 → 6)-linked β-D-galactofuranose units, or an acidic unit attached to the main chain through a D-galactofuranosyl linkage.

An α-(1 → 3)-linked glucan has been shown to occur[91] in *Cryptococcus*, *Schizosaccharomyces*, and *Polyporus* species, after extraction of the cell-walls with 3% sodium hydroxide at 75°. This procedure failed to extract the β-glucans present. Degradation of the α-glucan by an enzyme preparation from *Streptomyces* gave the disaccharide nigerose, 3-*O*-(α-D-glucopyranosyl)-D-glucose. It has been suggested that α-(1 → 3)-linked glucans are more widely distributed among the cell-walls of yeasts and fungi than had been suspected. A tetrasaccharide has been obtained from the hydrolysis of nigeran by the enzyme mycodextramase.[92] Partial acid hydrolysis, before and after reduction with sodium borotriti-ide, led to the conclusion that the tetrasaccharide had the structure (22). Incubation with mycodextranase gave nigerose as the sole product.

α-D-G*p*-(1 → 3)-α-D-G*p*-(1 → 4)-α-D-G*p*-(1 → 3)-D-G

(22)

The antigenic complex of a *Brucella abortus* variant has been divided into two polysaccharides. The first polysaccharide contained hexuronic acid, glucose, and xylose, whilst the second contained hexuronic acid, glucose, 2-amino-2-deoxyhexose, and an unidentified component.[93]

A polysaccharide (M_w 4400), limited in its distribution to the soluble portion of the cytoplasm, has been purified from the cell-free extracts of

[89a] L. P. T. M. Zevenhuizen, *Carbohydrate Res.*, 1968, **6**, 310.
[90] R. G. Brown and B. Lindberg, *Acta Chem. Scand.*, 1967, **21**, 2379.
[90a] R. G. Brown and B. Lindberg, *Acta Chem. Scand.*, 1967, **21**, 2383.
[91] J. S. D. Bacon, D. Jones, V. C. Farmer, and D. M. Webley, *Biochim. Biophys. Acta*, 1968, **158**, 313.
[92] K. K. Tung and T. H. Nordin, *Biochim. Biophys. Acta*, 1968, **158**, 31.
[93] I. I. Dubrovskaya and E. A. Dranovskya, *Biokhimiya*, 1967, **32**, 31.

Mycobacterium tuberculosis H37Ra.[94] The polysaccharide contained D-glucose (60%) and 6-*O*-methyl-D-glucose (40%) serving mostly as branch points in the backbone, and an acid group whose location has not as yet been ascertained. No evidence of covalent bonding to other cellular components was found. Isoiazid decreased the amount of alkali-extractable carbohydrate, mostly D-glucose and 6-*O*-methyl-D-glucose, obtainable from *M. tuberculosis*.[94a] An arabinogalactan (5 : 2) has been isolated from a strain of *M. tuberculosis* virulent in humans.[95] Methylation and periodate oxidation studies indicated that the polysaccharide contained (1 → 5)-linked and terminal non-reducing end groups of arabinose and (1 → 4)-linked galactose units. Four polysaccharide fractions have been recognised [95a, 95b, 96] in the culture filtrate of human tubercle bacilli strain H37Rv, an arabinogalactan (3 : 2, $[\alpha]_D$ +25·3°), an arabinomannan (1·8 : 1, $[\alpha]_D$ +58·3), a mannan ($[\alpha]_D$ +78·8°), and a glucan ($[\alpha]_D$ +155·0°). The arabinogalactan and arabinomannan were immunologically active and indistinguishable immunologically from those isolated from defatted cells of human tubercle bacilli strain Aoyama B. The glucan and mannan were not immunologically active. The arabinogalactan and arabinomannan elicited Arthus but not tuberculin-type skin reactions in sensitised animals.[95a] The culture filtrate of human tubercle bacilli strain Aoyama B yielded an arabinogalactan, $[\alpha]_D$ +31°, and a glucogalactomannan, $[\alpha]_D$ +70·5°.[96a] The latter polysaccharide showed no anaphylactic activity and little immunological activity. The arabinogalactan had analpylactic activity in guinea-pigs sensitised to heat-treated tubercle bacilli, but no tuberculin activity, and potential precipitin antigenicity. Skin reaction was lost on removal of protein, leading to the conclusion that pure polysaccharides yielded only an immediate-type immunologic reaction and had no capacity to elicit delayed-type skin reactions. D-Arabinose-5-mycollate has been isolated from *M. tuberculosis* strain Aoyama B.[97] The cell-wall polysaccharides of *M. tuberlosis* and *M. kansasii* have been shown to be identical by chemical and immunological investigations.[97a]

Mycobacterium phlei has been shown to contain a lipopolysaccharide of unknown function consisting of D-glucose, 3-*O*-methyl-D-glucose,6-*O*-methyl-D-glucose, and D-glyceric acid.[98] Digestion with α-amylase and glucamylase together with propylation studies indicated that the main

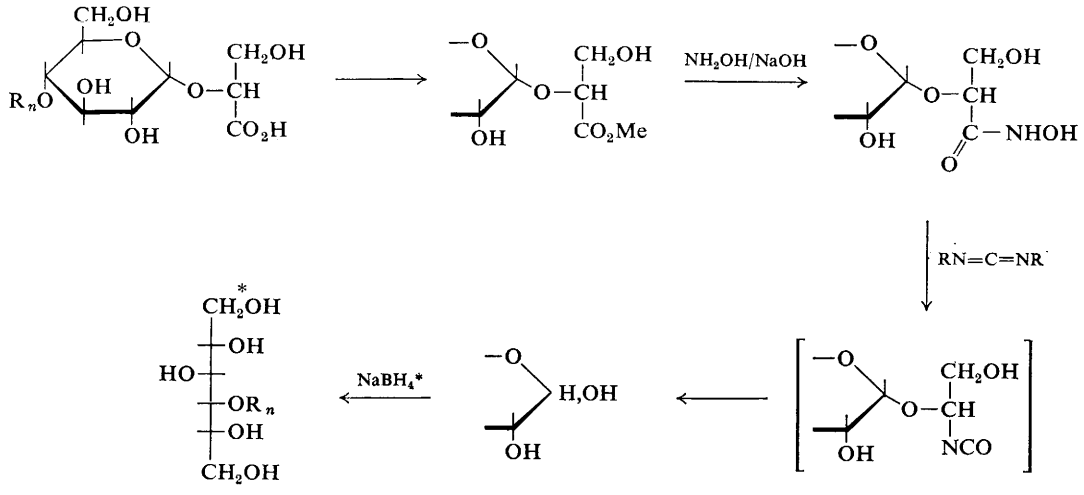
[94] F. A. Lornitzo and D. S. Goldman, *Biochim. Biophys. Acta*, 1968, **158**, 329.
[94a] F. G. Winder and S. A. Rooney, *Biochem. J.*, 1968, **110**, 8P.
[95] E. Vilkas, J. M. Delaumeny, and C. Nacasch, *Biochim. Biophys. Acta*, 1968, **158**, 147.
[95a] I. Azuma, H. Kimura, T. Niinaka, T. Aoki, and Y. Yamamura, *Jap. J. Microbiol.*, 1968, **12**, 367.
[95b] I. Azuma, H. Kimura, T. Niinaka, T. Aoki, and Y. Yamamura, *J. Bacteriol.*, 1968, **95**, 263.
[96] I. Azuma, H. Kimura, T. Niinaka, and Y. Yamamura, *J. Bacteriol.*, 1967, **93**, 770.
[96a] I. Azuma, H. Kimura, and Y. Yamamura, *Amer. Rev. Respirat. Dis.*, 1967, **96**, 536.
[97] I. Azuma, Y. Yamamura, and K. Fukushi, *J. Bacteriol.*, 1968, **96**, 1885.
[97a] S. E. Birnbaum and L. E. Affronti, *J. Bacteriol.*, 1968, **95**, 559.
[98] M. H. Saier jun. and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 992.

polysaccharide chain consisted of α-(1 → 4)-linked 6-*O*-methyl-D-glucose residues with α-(1 → 3)- branch points. Non-reducing terminal residues were single D-glucose units and 3-*O*-methyl-D-glucose units attached to a short section of α-(1 → 4)-linked D-glucose units. Conversion of the polysaccharide to the hydroxamate, followed by a Lossen rearrangement (23) in aqueous solution at a neutral pH, showed that the glyceric acid was glycosidically attached to a D-glucose residue. A complete structure for the polysaccharide (24) could be formulated from the results of methylation and periodate oxidation studies on the original polysaccharide, the products from enzyme degradation and the product from the Lossen rearrangement.[99, 99a] The lipopolysaccharide was resolved into four components which differed from each other in total charge owing to the presence of 0, 1, 2, or 3 monoesterified succinate residues.[100] In addition each component contained four other acyl groups identified as acetate, propionate, *iso*-butyrate, and octanate (3 : 1 : 1 : 1). This was the first report of *iso*-butyrate, propionate, or octanate occurring as a substituent of a natural carbohydrate polymer. The same lipopolysaccharides have been isolated from *M. tuberculosis* H37Ra, and *M. smegmatis*. *M. tuberculosis* (lederle) yielded a single form of the lipopolysaccharide which contained only one acyl group, octanate, although the latter could have been an artefact.[100]

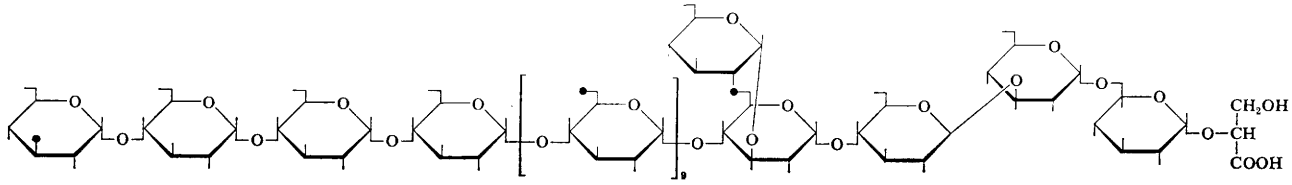
Structural studies on the O-specific side-chains of the cell-wall lipopolysaccharide from *Salmonella typhimurium* 395MS have given results with important implications in regard to the immunochemistry of the O-factor.[101] Methylation of the original polysaccharide, using methyl sulphinylcarbanion–methyl iodide–DMSO, and of a mild acid-hydrolysis product enabled a structure to be formulated (25). The location of the *O*-acetyl group was established, as the C-2-position of abequose (3,6-dideoxy-D-*xylo*-hexose), by methylation after prior acetalation (methyl vinyl ether, toluene-*p*-sulphonic acid, DMSO) and alkaline deacetylation. This polysaccharide contained the O-factors 4, 5, and 12. Originally the O-factor 5 had been thought to be due to an *O*-acetyl group at the C-2 of the D-galactose residues as a result of inhibition studies with 2-acetamido-2-deoxy-D-galactose, but this was not apparently the case in the strain 395MS.[101] The chemical composition[102] and immunological cross-reactions[102a] of the lipopolysaccharides from rough mutants of *S. typhimurium* have been investigated.

The chemical changes occurring in the specific polysaccharide of *S. cholerae suis* (O-antigen type 6_2 and 7) after its conversion by phage

[99] M. H. Saier jun. and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 4332.
[99a] M. H. Saier jun. and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 4319.
[100] J. M. Keller and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 2905.
[101] C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Carbohydrate Res.*, 1968, **8**, 43.
[102] A. A. Lindberg and T. Holme, *J. Gen. Microbiol.*, 1968, **52**, 55.
[102a] T. Holme, A. A. Lindberg, P. J. Garegg, and T. Onn, *J. Gen. Microbiol.*, 1968, **52**, 45.



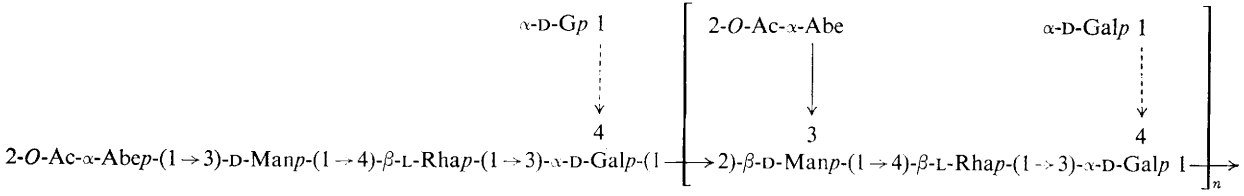
(23)



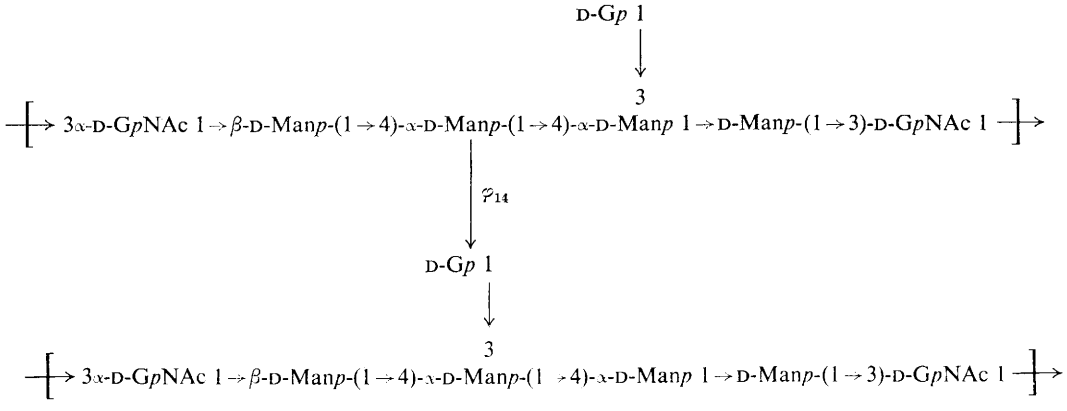
• = Me

(24)

Positions of ester substituents are not known, acetate (3 moles), propionate (1), isobutyrate (1), succinate (0·3), and actonate (1).


α-D-Gp 1
2-O-Ac-α-Abe
α-D-Galp 1
4
3
4
2-O-Ac-α-Abep-(1→3)-D-Manp-(1→4)-β-L-Rhap-(1→3)-α-D-Galp-(1→2)-β-D-Manp-(1→4)-β-L-Rhap-(1→3)-α-D-Galp 1
n
n = ca. 10

(25)


D-Gp 1
3
3α-D-GpNAc 1→β-D-Manp-(1→4)-α-D-Manp-(1→4)-α-D-Manp 1→D-Manp-(1→3)-D-GpNAc 1
φ14
D-Gp 1
3
3α-D-GpNAc 1→β-D-Manp-(1→4)-α-D-Manp-(1→4)-α-D-Manp 1→D-Manp-(1→3)-D-GpNAc 1

(25a)

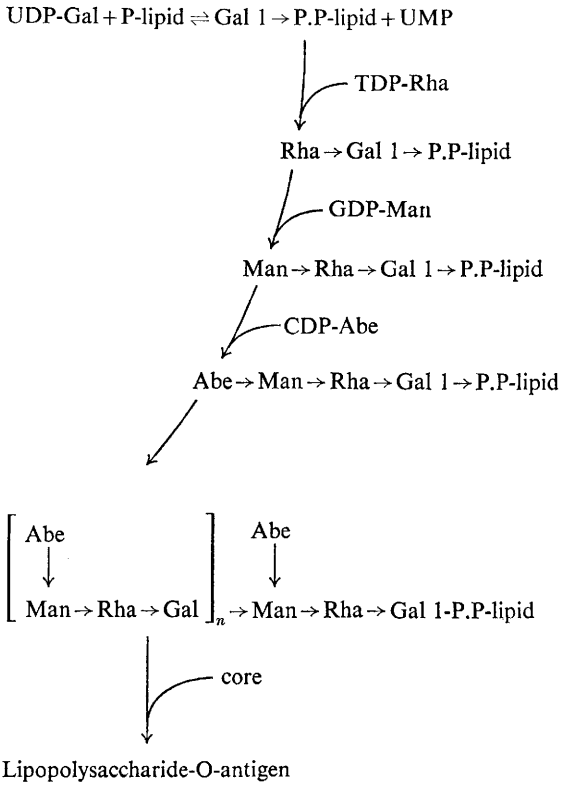
14 [O-antigen 6_2, (7) and (14)] have been elucidated.[103] The D-glucopyranosyl substituent residues were displaced to an adjacent D-mannopyranosyl unit in the main chain in the phage 14-converted polysaccharide (25a). Chemical and immunochemical studies of the lipopolysaccharides of *Salmonella* R mutants [104] and T1, S hybrids of *S. paratyphi*-B [105] have been reported. 2-Amino-2-deoxy-D-mannose has been identified in hydrolysates of the lipopolysaccharides of *Salmonella* groups J and T, *E. coli* O31, and *Arizona* 15, whilst 2-amino-2,6-dideoxyglucose was obtained from those of *Salmonella* groups S and 58, *Arizona* 1·33, and *Proteus vulgaris*.[106]

Additional evidence has been furnished for the participation of lipid-linked intermediates in the synthesis and polymerisation of the tetrasaccharide repeating unit of the lipopolysaccharide from *S. typhimurium*.[107, 108] A particulate cell envelope fraction from *S. typhimurium* catalysed the enzymic incorporation of abequose into the repeating unit of the O-antigen. The initial step was the formation of a lipid-linked tetrasaccharide which has been isolated and characterised. The growing O-antigen remained attached to the lipid during the polymerisation stages, and the resultant enzymatically synthesised polysaccharide was shown to correspond to that of the authentic O-antigen. The evidence obtained enabled a reaction sequence (26) to be postulated.

A study of the cell-wall lipopolysaccharides of *Neisseria perflava* suggested that the backbone of the lipopolysaccharide was very similar to that found in the *Salmonella–Escherichia* group.[109] Both chloroform-soluble and -insoluble fractions contained 3-deoxyoctulosonic acid (KDO), lipid A, glucose, rhamnose, heptose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, ethanolamine, and fatty-acid components, but differed markedly in the proportions of KDO to neutral sugars and neutral sugars to fatty acids. Ethanolamine was probably linked to the C-6-hydroxy-group of 2-amino-2-deoxyglucose and the fatty acids bound by both amide and ester linkages. The heptose units were linked linearly ($1 \rightarrow 3$) as were 25% of the glucose units. Other glucose units and the rhamnose units were present as branching points.

A capsular polysaccharide, $[\alpha]_D$ $-40°$, has been isolated from *Achrombacter georgiopolitanum*.[110] The polysaccharide, which was antigenic,

[103] N. A. Fuller and A. M. Staub, *European J. Biochem.*, 1968, **4**, 286. N. A. Fuller, M. Etievant, and A. M. Staub, *European J. Biochem.*, 1968, **6**, 525.
[104] W. Droge, E. Ruschmann, O. Luderitz, and O. Westphal, *European J. Biochem.*, W. Droge, O. Luderitz, and O. Westphal, *European J. Biochem.*, 1968, **4**, 126; E. Mikulaszek, B. Kedzierska, and J. Pogonowska-Goldhar, *Bull. Acad. Pol. Sci. Biol.*, 1967, **15**, 665.
[105] M. Sarvas, O. Luderitz, and O. Westphal, *Ann. Med. Exptl. Biol. Fenniae*, 1967, **45**, 117.
[106] O. Luderitz, J. Gmeiner, B. Kickhofen, H. Mayer, O Westphal, and R. W. Wheat, *J. Bacteriol.*, 1968, **95**, 40.
[107] M. J. Osborn and I. M. Weiner, *J. Biol. Chem.*, 1968, **243**, 2631.
[108] M. J. Osborn and R. Y. Tze-Yuen, *J. Biol. Chem.*, 1968, **243**, 5145.
[109] G. A. Adams, M. Kates, D. H. Shaw, and M. Yaguchi, *Canad. J. Biochem.*, 1968, **46**, 1171.
[110] E. J. Smith, *J. Biol. Chem.*, 1968, **243**, 5139.



(26)

contained 2-acetamido-2-deoxy-D-glucuronic acid, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2,6-dideoxy-D-glucose (2 : 1 : 2). The polysaccharide chain consisted of 2-acetamido-2-deoxy-D-glucose analogues with either an equatorial carboxy group or an equatorial methyl group attached to C-5. The non-encapsulated strain of this organism accumulated UDP-2-acetamido-2-deoxy-D-glucuronic acid.[111]

The structure of the extracellular polysaccharide from *Arthrobacter viscosus* NRRL B-1973 has been investigated.[112] Acid hydrolysis of the polysaccharide gave equimolar amounts of D-glucose, D-galactose, and D-mannuronic acid (accounting for 75% of the dry weight of the polysaccharide), the remainder being present as *O*-acetyl groups. This corresponded to 50% of the theoretical value for total acetylation. Periodate

[111] E. J. Smith, *Biochim. Biophys. Acta*, 1968, **158**, 470.
[112] J. H. Sloneker, D. G. Orentas, C. A. Knutson, P. R. Watson, and A. Jeanes, *Canad. J. Chem.*, 1968, **46**, 3353.

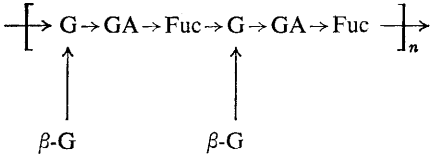
oxidation of the native and deacetylated material indicated a linear structure consisting predominantly of repeating trisaccharide units (27) with four

$$\beta\text{-D-Man}p\text{A-}(1\rightarrow4)\text{-}\beta\text{-D-G}p\text{-}(1\rightarrow4)\text{-D-Gal}$$

(27)

acetyl groups per trisaccharide. It is possible that 10–12% of the D-glucose residues were glycosidically substituted at C-3 and that 20% of the D-mannuronic acid units were substituted at C-3 or existed as a 6,3-lactone. *Ca.* 80% of the glucose residues in the deacetylated polysaccharide were resistant to periodate oxidation and apparently substituted at C-3. However, methylation studies [113] of the polymer as well as of the aldotriuronic acid provided unequivocal evidence that the resistant residues were substituted at C-4 by a D-mannuronosyl residue. It was proposed that the carbonyl at C-2 and C-3 of the more rapidly oxidised D-mannuronosyl acid and D-galactose residues respectively were in excellent positions to form six-membered cyclic hemiacetals with the hydroxy-groups at C-3 and C-4 of the unoxidised D-glucose residues. These results emphasised the general need for caution in the interpretation of the periodate oxidation data.

Further studies have been reported on the exopolysaccharide of *Klebsiella aerogenes* A3(S1) Type 54.[114] The polysaccharide contained glucose, glucuronic acid, fucose, and acetyl groups (4 : 2 : 2 : 1). A phage-induced fucosidase hydrolysed the polysaccharide to an octasaccharide, considered to be the repeating unit since this contained the same proportions of the components as in the original polysaccharide. This octasaccharide was further hydrolysed by other phage-induced fucosidases to two tetrasaccharides differing only in that one was acylated. From this an acylated trisaccharide was obtained by degradation with a commercial 'cellulase'. From the evidence obtained a possible repeating unit (28) was postulated.



(28)

The separation and chemical composition of the lipopolysaccharides of *Aerobacter aerogenes* strains A3(S1) and NCTC 243 have been reported.[114a]

Seven mutant strains of *Alcaligenes faecalis* var. *myxogenes* 10C3 produced by a mutagen, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, were

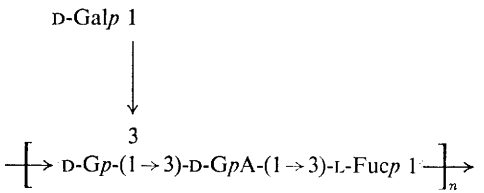
[113] I. R. Siddiqui, *Carbohydrate Res.*, 1967, **4**, 277.
[114] I. W. Sutherland and J. F. Wilkinson, *Biochem. J.*, 1968, **110**, 749.
[114a] D. E. Koeltzow, J. D. Epley, and H. E. Conrad, *Biochemistry*, 1968, **7**, 2920.

isolated, and their ability to produce a succinoglucan and curdlan (a gel-forming polysaccharide) was investigated.[115] Structural studies [115a] showed that curdlan was a β-(1 → 3)-linked D-glucan of mean degree of polymerisation ($\overline{DP}$) 135.

Cell extracts of *Streptococcus mitis* contained a pullulanase and a transglycosylase.[116] The pullulanase acted rapidly on α-(1 → 6)-linkages in substrates having the structure α-maltotriosyl-(1 → 6)-maltodextrin. The enzyme did not degrade isomaltose, 6-D-glucosyl maltodextrins, or 6-α-maltodextrinyl-D-glucoses, and appeared to have a minimum requirement of 6^2-α-maltosylmaltose. The branch linkages of amylopectin phosphorylase limit dextrin, glycogen phosphorylase limit dextrin, and glycogen β-amylase limit dextrin were hydrolysed by the pullulanase.

3-Deoxy-D-*manno*-octulosonic acid has been found in the lipopolysaccharide of *Pasteurella* species and it appeared that this compound was as widely distributed in the lipopolysaccharides of the *Brucellaceae* as in the *Enterobacteriaceae*.[117]

A mutant (J5) of *Escherichia coli* produced a cell-wall lipopolysaccharide which did not contain galactose, glucose, 2-acetamido-2-deoxyglucose, or colitose.[118] It was demonstrated that the presence or absence of specific sugars in the lipopolysaccharide was a determinant of its antiphagocytic capacity and its virulence.[118] A study of the heptoses of lipopolysaccharide from Gram-negative bacteria [118a] revealed that the relative amounts of D-*glycero*- and L-*glycero*-D-*manno*-heptose varied widely among the various species. Both heptoses were isolated and characterised from a strain of *E. coli*. The K-antigen of *E. coli* 08:K27(A):H$^-$ contained D-glucuronic acid, D-glucose, D-galactose, and L-fucose in equimolar proportions together with *O*-acetyl functions (5·2%).[119] Structural investigations allowed a possible repeating unit (28a) to be formulated. The relationship between



(28a)

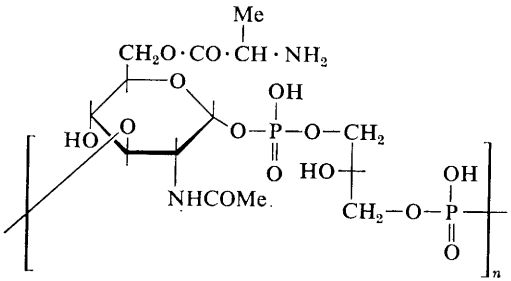
[115] T. Harada, A. Amemura, H. Saito, S. Kanamaru, and A. Misaki, *J. Ferment. Technol.*, 1968, **46**, 679.
[115a] T. Harada, A. Misaki, and H. Saito, *Arch. Biochem. Biophys.*, 1968, **124**, 292.
[116] J. G. Walker, *Biochem. J.*, 1968, **108**, 33.
[117] D. C. Ellwood, *Biochem. J.*, 1968, **106**, 47P.
[118] D. N. Medearis jun., B. C. Camilta, and E. C. Heath, *J. Exptl. Med.*, 1968, **128**, 399.
[118a] G. A. Adams, C. Quadling, and M. B. Perry, *Canad. J. Microbiol.*, 1967, **13**, 1605.
[119] K. Jahn, B. Jahn, K. Schneider, F. Ørskov, and I. Ørskov, *European J. Biochem.*, 1968, **5**, 456.

the structures of the K30, K42, and K85 polysaccharides of *E. coli* and the cross-reactivity in antipneumococcal sera has been investigated.[120]

The chemical composition of polysaccharides from *Xanthomonas* species [121] and *Citrobacter freundii* [121a] have been reported.

Bacterial Cell-walls

Further studies have been reported of the structure and biosynthesis of the complex molecular architecture of bacterial cell-walls. A glycerol teichoic acid containing phosphate, D-alanine, and D-glucose (1 : 0·25 : 0·5) has been extracted from the cell-walls of *Staphylococcus epidermidis* I2.[122] This teichoic acid was shown to be a (1 → 3)-linked poly(glycerol phosphate) with β-D-glucopyranosyl and D-alanyl ester substituents. 2-*O*-β-D-Glucosylpyranosyl glycerol was isolated and characterised. The membrane teichoic acid was also a (1 → 3)-linked poly(glycerol phosphate) but contained a smaller proportion of glucosyl substituents. The glycerol teichoic acid from the cell-walls of *Streptococcus lactis* I3 contained glycerol, 2-acetamido-2-deoxy-D-glucose, phosphate, and D-alanine (1 : 1 : 2 : 1) with the alanine residues attached *via* ester linkages.[123] Mild acid-hydrolysis yielded the repeating unit (29), whilst hydrolysis with alkali liberated glycerol diphos-



(29)

phate, metasaccharinic acid (3-deoxyhexonic acid), and two phosphodiesters containing 2-amino-2-deoxy-D-glucose. Additional structural features were established from the results of periodate oxidation studies. The membrane teichoic acid of *Staphylococcus lactis* I3 was similar to the membrane teichoic acid from *S. epidermis* I2, in that it consisted of a (1 → 3)-linked poly(glycerol phosphate) with alkali-labile alanine ester substituents, but contained little or no sugar or amino-sugar substituents.[124] The *S. lactis*

[120] M. Heidelberger, K. Jahn, B. Jahn, F. Ørskov, I. Ørskov, and O. Westphal, *J. Bacteriol.*, 1968, **95**, 2415.
[121] W. A. Volk, *J. Bacteriol.*, 1968, **95**, 980.
[121a] R. A. Raff and R. W. Wheat, *J. Bacteriol.*, 1968, **95**, 2035.
[122] A. R. Archibald, J. Baddiley, and G. A. Shaukat, *Biochem. J.*, 1968, **110**, 583.
[123] A. R. Archibald, J. Baddiley, and D. Button, *Biochem. J.*, 1968, **110**, 543.
[124] A. R. Archibald, J. Baddiley, and D. Button, *Biochem. J.*, 1968, **110**, 559.

membrane teichoic acid was structurally different from the cell-wall teichoic acid of the same organism. *Micrococcus* (*Staphylococcus*) *lactis* appeared to contain an atypical cell-wall glycerol teichoic acid, different fundamentally from all other examples previously encountered.[125] Study of the cell-wall and membrane teichoic acids in *Staphylococci* and *Micrococci* indicated that all contained membrane teichoic acids in which the polyol was glycerol. However, the presence and structure of the cell-wall teichoic acids were characteristic of the different species.[125] Biosynthetic studies indicated that glycerol phosphate was derived from CDP-glycerol, and 2-acetamido-2-deoxy-D-glucose-1-phosphate from UDP-2-acetamido-2-deoxy-D-glucose.[126] No polymer was synthesised unless both nucleotides were present, but no other substrates were required. UDP-2-Acetamido-2-deoxy-D-glucose donated its 2-acetamido-2-deoxy-D-glucose-1-phosphate moiety as an intact unit to the polymer.

Micrococcus lysodeikticus grown in a defined medium in the presence of D-serine incorporated this amino-acid into the mucopeptide whilst glycine incorporation was decreased.[127] It was suggested that *ca.* 75–80% of the incorporated serine could substitute for glycine and was attached *via* its amino-group to the α-carboxyl group of glutamic acid. Glycyl transfer RNA synthetase[128] and three glycine acceptor transfer RNA species[129] have been isolated from *S. aureus*. The incorporation of L-threonine into interpeptide bridges in *Micrococcus roseus*,[130] and of serine and glycine into interpeptide bridges in *S. epidermidis*[131] cell-wall peptidoglycans, have been studied in particulate enzyme systems. The lipid intermediates in peptidoglycan synthesis in *S. aurens*, *N*-acetylmuramyl-(pentapeptide)-P-P-lipid and 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramyl-(pentapeptide)-P-P-lipid acted as acceptors of ammonia in an ATP-dependent reaction in which the α-carboxyl group of glutamic acid was amidated.[132] The cross-linking in the peptidoglycan of *S. aureus* was inhibited by penicillins and cephalosporins in an *in vivo* study.[133]

The glycerol teichoic acid of the cell wall of *Actinomyces antibioticus*, containing phosphate, galactose, and 2-acetamido-2-deoxy-D-galactose (0·8 : 1·08 : 1·0), has been investigated.[134] Alkaline and subsequent phosphomonoesterase hydrolysis enabled the polymer to be formulated as consisting of D-galactose linked (1 → 3 or 4) to 2-acetamido-2-deoxy-D-galactosyl-glycerol linked *via* phosphodiester bonds in the C-1 and C-2 position of glycerol.

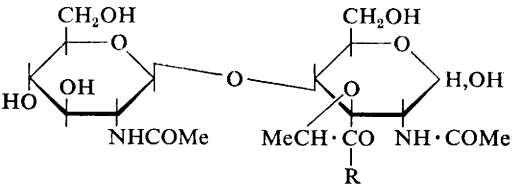
[125] A. L. Davidson, *Biochem. J.*, 1968, **110**, 557.
[126] J. Baddiley, N. L. Blumson, and L. J. Douglas, *Biochem. J.*, 1968, **110**, 565.
[127] J. G. Whitney and E. A. Grula, *Biochim. Biophys. Acta*, 1968, **158**, 124.
[128] B. Niyomporn, J. L. Dahl, and J. S. Strominger, *J. Biol. Chem.*, 1968, **243**, 773.
[129] R. M. Bumsted, J. L. Dahl, D. Söll, and J. L. Strominger, *J. Biol. Chem.*, 1968, **243**, 779.
[130] W. S. L. Roberts, J. L. Strominger, and D. Söll, *J. Biol. Chem.*, 1968, **243**, 749.
[131] J. F. Petit, J. L. Strominger, and D. Söll, *J. Biol. Chem.*, 1968, **243**, 757.
[132] G. Siewert, and J. L. Strominger, *J. Biol. Chem.*, 1968, **243**, 783.
[133] D. J. Tipper and J. L. Strominger, *J. Biol. Chem.*, 1968, **243**, 3169.
[134] M. Sh. Zaretskaya, I. B. Naumova, and Z. A. Shabarova, *Biokhimiya*, 1967, **32**, 796.

Bacillus subtilis var. *niger* cell-walls contained a teichoic acid composed of glycerol phosphate and glucose, but when this organism was grown in a chemostat with a phosphate-limiting medium the cell-wall teichoic acid was completely displaced by a phosphate-free acidic polymer of 2-acetamido-2-deoxy-D-galactose and glucuronic acid (*i.e.* a teichuronic acid).[135] With *Bacillus subtilis* W23 the ribitol teichoic acid was totally displaced by a teichuronic acid polymer. 'Intracellular' teichoic acids were present however, even in the phosphate-limited organisms. Intracellular production of mucopeptide precursors continued in Mg^{2+}-deprived *B. subtilis* but the later stages of cell-wall synthesis, in which membrane-bound carriers and enzymes were involved, were inhibited.[136] An amino-sugar of unknown structure accumulated in the acid-soluble fraction of *B. subtilis* strain 83 during treatment with penicillin, and the mucopeptides in cells treated with penicillin possessed a characteristically lower muramic acid content.[137]

A disaccharide, and two tripeptide derivatives of this disaccharide have been isolated and characterised from lysozyme digests of *B. lichenformis* ATCC 9945 cell-walls.[138] The disaccharide was identified as *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (30). The tripeptide derivatives (31 and 32) contained an amide bond between the carboxyl group of muramic acid and the amino terminal group of the tripeptide. Small amounts of a tetrasaccharide (33) were also isolated. These two disaccharide tripeptides (31 and 32) have been isolated from *B. lichenformis* NCTC 6346.[139] The action of dilute alkali on some bacterial cell-walls has been studied.[140] The cell-walls of *B. stearothermophilus* B65 contained a glycerol teichoic acid which was probably covalently linked to peptidoglycan through muramic acid phosphate.[141] A simple method for the preparation of the cell-wall intermediate UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-2,6-diaminopimelic acid and its characterisation in *B. cereus* has been described.[142]

A polysaccharide component of the cell-walls of *Lactobaccillus fermenti* consisted of glucose and galactose, and it was suggested that the intact wall polysaccharide was joined to muramic acid *via* a phosphodiester linkage.[143] Cell-walls of *Rhodospirillum rubrum* contained additional peptide cross-linkages not present in bacilliform mutant cells.[144] It was concluded that the bacilliform mutants were bradytrophic in some way concerning the synthesis of D-alanine, necessary to form the requisite precursors for peptidoglycan cross-linkages.

[135] D. C. Ellwood and D. W. Tempest, *Biochem. J.*, 1968, **108**, 40P.
[136] A. J. Garrett, *Biochem. J.*, 1968, **106**, 40P.
[137] L. I. Tarbochkima and I. N. Navol'neva, *Biokhimiya*, 1967, **32**, 705.
[138] D. Mitelman and N. Sharon, *J. Biol. Chem.*, 1968, **243**, 2279.
[139] R. C. Hughes, *Biochem. J.*, 1967, **102**, 26P.
[140] R. C. Hughes and P. S. Tanner, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 22.
[141] W. D. Grant and A. J. Wicken, *Biochem. Biophys. Res. Comm.*, 1968, **32**, 122.
[142] T. Nakatini, Y. Araki, and E. Ito, *Biochim. Biophys. Acta*, 1968, **156**, 210.
[143] K. W. Knox and K. J. Holmwood, *Biochem. J.*, 1968, **108**, 363.
[144] J. W. Newton, *Biochim. Biophys. Acta*, 1968, **165**, 534.



(30) R = OH

(31) R = NH—CH(Me)—CO—NH—CH(CO_2H)—$(CH_2)_2$—CO—NH—CH(CO_2H)—$(CH_2)_3$—CH(CO_2H)—NH_2

(32) R = NH—CH(Me)—CO—NH—CH($CONH_2$)—$(CH_2)_2$—CO—NH—CH(CO_2H)—$(CH_2)_3$—CH(CO_2H)—NH_2

L-ala D-glut *meso*-DAP

β-D-G*p*NAc-(1→4)-β-MurNAc-(1→4)-β-D-G*p*NAc-(1→4)-MurNAc

(33)

Chemical analyses have been performed on the cell-walls of *Mycobacterium tuberculosis* BCG, *M. smegmatis*, and related strains.[145] The cell-wall fraction contained the species specific mycolis acid (35–40%), a D-arabino-D-galactan (33–40%), and a mucopeptide (19–26%) containing 2-amino-2-deoxyglucose and muramic acid. Mycolic acid was attached to the arabinogalactan *via* ester linkages, and the arabinogalactan was probably linked to the mucopeptide *via* phosphatediester linkages. It was suggested that the mycolic acid–arabinogalactan–mucopeptide complex was a structure common to mycobacterial cell-walls. The size, and also the nature, of the polysaccharide portion in a peptidoglycolipid of *Mycobacteria* was possibly significant for adjuvant activity.[146]

D-Alanine carboxypeptidase and peptidoglycan transpeptidase from *Escherichia coli* have been studied with respect to the biosynthesis of cell-wall peptidoglycan.[147, 148]

[145] F. Kanetsuna, *Biochim. Biophys. Acta*, 1968, **158**, 130.
[146] T. Koga, T. Ishibashi, K. Sugiyama, and A. Tanaka, *Biochim. Biophys. Acta*, 1968, **158**, 144.
[147] K. Izaki and J. L. Strominger, *J. Biol. Chem.*, 1968, **243**, 3193.
[148] K. Izaki, M. Matsuhashi, and J. L. Strominger, *J. Biol. Chem.*, 1968, **243**, 3180.

Sub-unit peptides of the peptidoglycan from cell-walls of three Gram-positive bacteria have been synthesised to corroborate peptides obtained by degradative studies.[149]

Further studies have been reported on the specificity and mode of action of lysozyme.[149a]

Fungal Polysaccharides

Further structural studies have been reported of polysaccharides isolated from a range of fungi, and in certain cases the biological properties of such materials have been investigated.

Methylation studies confirmed that pustulan, a glucan from the lichen *Umbilicaria pustulata*, was essentially a β-(1 → 6)-linked glucan.[150] Extraction of alkali-extracted yeast with acetic acid gave a water-soluble, β-(1 → 6)-linked, glucan in addition to glycogen.[151] It was suggested that this glucan accounted for the discrepancies in proposed structures for yeast glucan.

Cultivation of the rot fungus *Stereum sanguinolentum* in a medium containing cellulose as the sole carbon source resulted[152] in the formation of an extracellular D-glucan ($[\alpha]_D$ +10°, in 1M-KOH). Products of methylation were identified by mass spectroscopic analysis of the alditol acetates. The polysaccharide appeared to consist of a β-(1 → 3)-linked D-glucan backbone with branching at the C-6 position on *c.a.* every third unit. The branches were probably short, possibly consisting of single D-glucose units.

A more complex D-glucan, $[\alpha]_D$ −10° in DMSO, from the cell wall of *Saccharomyces cerevisiae* has been examined.[153] Periodate oxidation and methylation studies indicated that the polysaccharide consisted of a β-(1 → 3)-linked D-glucan with branches at C-6, with a small proportion of β-(1 → 6)-linked units. Mild acid hydrolysis of the product from periodate oxidation and borohydride reduction resulted in the loss of one glucose unit out of nine, to give a glucan of $\overline{DP}$ 150 (*cf.* $\overline{DP}$ 410 for the original glucan). Degradation of both the intact glucan and degraded glucan with the (1 → 3)-β-D-glucanase from *Rhizopus arrhizus* gave the same products, D-glucose, laminaribiose, gentiobiose, and higher oligosaccharides. A working model for the structure of the glucan (34) was proposed.

[149] P. Lefrancier and E. Bricas, *Bull. Soc. Chim. Biol.*, 1967, **49**, 1257.

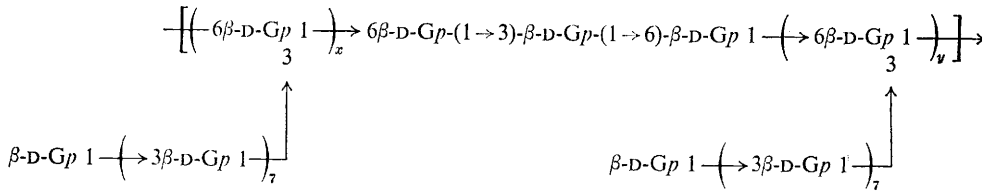
[149a] G. Lowe and G. Sheppard, *Chem. Comm.*, 1968, 529; M. A. Raftery, F. W. Dahlqvist, S. I. Chan, and S. M. Parsons, *J. Biol. Chem.*, 1968, **243**, 4175; U. Zehavi, J. J. Pollock, V. Teichberg, and N. Sharon, *Israel J. Chem.*, 1968, **6**, 119P; J. J. Pollock, U. Zehavi, V. Teichberg, and N. Sharon, *Israel J. Chem.*, 1968, **6**, 120P; D. M. Chapman, J. J. Pollock, and N. Sharon, *J. Biol. Chem.*, 1968, **243**, 487; U. Zehavi, J. J. Pollock, V. Teichberg, and N. Sharon, *Nature*, 1968, **219**, 1152.

[150] C. G. Hellerqvist, B. Lindberg, and K. Samuelson, *Acta Chem. Scand.*, 1968, **22**, 2736.

[151] J. S. D. Bacon and V. C. Farmer, *Biochem. J.*, 1968, **110**, 34P.

[152] K. Axelsson, H. B. Bjorndal, and K. E. Erikson, *Acta Chem. Scand.*, 1968, **22**, 1363.

[153] A. Misaki, J. Johnson jun., S. Kirkwood, J. V. Scaletti, and F. Smith, *Carbohydrate Res.*, 1968, **6**, 150.



$x + y = 40\text{–}50$

(34)

The 'laminarin' of *Ishige okamurai* Yendo was examined [154] in order to compare its structure with that of laminarins of the *Laminariales*, a far remote family. Periodate oxidation, methylation, and partial acid hydrolysis studies indicated the presence of β-(1 → 3)- and β-(1 → 6)-linked units (*c.a.* 1 : 4), and in addition some glucose units substituted at both C-3 and C-6. The polysaccharide appeared similar to that from *Eisenia bicyclis* Setchell (N. Handa and K. Nisizawa, *Nature*, 1961, **192**, 1078), although containing different proportions of linkages. No mannitol was found in acid hydrolysates.

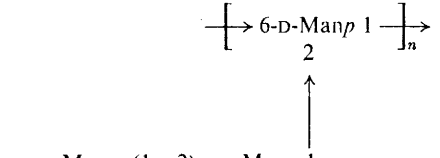
Experimental evidence has been provided for the presence of a mixture of both iso-amylase and amylo-1,6-glucosidase in the α-(1 → 6)-glucosidase from baker's yeast.[154a]

A glucogalactan (1 : 2) from the yeast-like fungus *Sporobolomyces roseus* has been investigated.[155] Smith degradation indicated that 60% of the hexose units were terminal non-reducing or (1 → 6)-linked and 10% were resistant to periodate oxidation, these being exclusively galactose units. No (1 → 4)-linked units were postulated, the remaining 30% of the hexose content being (1 → 2)-linked. The immunological cross-reactions of acetylphosphogalactans from *Sporobolomyces* with antipneumococcal and antisalmonella sera have been studied.[155a]

The major water-soluble polysaccharide of the mycelium from *Penicillium chrysogenum* was a galactomannan (D-galactose : D-mannose; 2 : 3) containing traces of glucose.[156] Periodate oxidation and Smith degradation suggested a linear structure with (1 → 2)-linkages and (1 → 3)-linkages as predominating features. In addition, (1 → 4)-linked galactopyranose or (1 → 5)- or (1 → 6)-linked galactofuranose residues were found. A galactoglucomannan, isolated from *Penicillium charlesii* var. *Smith*, has been reported to contain β-(1 → 5)-linked D-galactofuranose units.[156a] Alkali-soluble galactomannans formed by certain species of the genera *Trichosporon*, *Schizosaccharomyces*, and *Nadsoma* have been studied by n.m.r.

[154] M. Maeda and K. Nisizawa, *Carbohydrate Res.*, 1968, **7**, 97.
[154a] G. N. Bathgate and D. J. Manners, *Biochem. J.*, 1968, **107**, 443.
[155] N. P. Elinov and G. A. Vitivskaya, *Biokhimiya*, 1967, **32**, 337.
[155a] M. Heidelberger and M. E. Slodki, *J. Exptl. Med.*, 1968, **128**, 189.
[156] T. Miyozaki and T. Yadomae, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 1721.
[156a] J. F. Preston and J. E. Gander, *Arch. Biochem. Biophys.*, 1968, **124**, 504.

spectroscopy and chemical methods.[157] Different species of *Trichosporon* formed galactomannans, pentosylmannans, and a mannan. The galactomannans were distinguishable from those of *Schizosaccharomyces* by the 1-H region of their n.m.r. spectra at 70° in deuterium oxide. Spectra of *T. hellenicum* and *N. fulvescens* galactomannans were virtually indistinguishable. The galactomannan (13 : 7) from *T. fermentans* was assigned a main structure (35) on the basis of methylation, Smith degradation,



(35)

partial acetolysis, and acid hydrolysis data. Presumed removal of galactofuranose units from side-chains of the galactomannan of *Aspergillus fumigatus* by partial acid hydrolysis resulted [158] in loss of the homologous precipitin activity. Growth of *Lipomyces starkeyi* on a glucose substrate produced a slime of polysaccharide which gave an insoluble galactomannan copper complex and a soluble 'starch-like' polysaccharide.[158a] The galactomannan (2 : 5), of $\overline{DP}$ 211 and $[\alpha]_D$ +41°, contained β-(1 → 6)-linked units in a backbone with (1 → 2)-linked side-chains on the evidence of methylation and periodate oxidation studies. For every five mannose units in the main chain three were branch points, one was substituted with a non-reducing mannose terminal unit, and the others with (1 → 2)-linked galactose units of average chain length two units. 6-*O*-α-D-Mannopyranosyl-D-mannose and 2-*O*-β-D-galactopyranosyl-D-galactose were isolated from a partial hydrolysis of the polysaccharide with formic acid.

The two extracellular polysaccharides of *Candida bogoriensis* contained[159] D-mannose (1), L-fucose (1·8), L-rhamnose (1·36), D-glucoronic acid (neutral equivalent 1580), and D-galactose (0·80). Structural studies were performed on the mixed polysaccharides. The main heteropolymer (> 80%) had α-D-(1 → 3)-linked mannose units in a main chain from the results of Smith degradation. Partial acid hydrolysis gave a series of disaccharides (36–39) arising from side-chains. Further evidence was

(36) 3-*O*-α-L-Rha*p*-L-Rha
(37) 2-*O*-α-L-Rha*p*-L-Fuc
(38) 3-*O*-α-L-Fuc-L-Fuc
(39) 4-*O*-β-D-G*p*A-L-Fuc
(40) α-L-Rha*p*-(1 → 4)-β-D-G*p*A-(1 → 4)-Fuc

[157] P. A. J. Gorin and J. F. T. Spencer *Canad. J. Chem.*, 1968, **46**, 2299.
[158] O. Sakaguchi, M. Suzuki, and K. Yokota, *Jap. J. Microbiol.*, 1968, **12**, 123.
[158a] O. Šikl, L. Masler, and Š. Bauer, *Collect. Czech. Chem. Commun.*, 1968, **33**, 1157.
[159] P. A. J. Gorin and J. F. T. Spencer, *Canad. J. Chem.*, 1968, **46**, 3407.

obtained for a partial sequence (40) of units in the polysaccharides. The specificity and immunogenicity of soluble polysaccharides from *Candida albicans* were studied using agglutinins of a IgG nature.[159a] The data obtained suggested that IgG produced by immunisation with the soluble polysaccharide contained a specific antibody factor capable of differentiating *C. albicans* from *C. tropicalis*. The nature of the cross-reaction of the mycelial mannan of *Trichophyton rubrum* and a galactomannan isolated from the culture medium of *Aspergillus fumigatus* with antisera of *Saccharomyces cerevisiae* and *Candida albicans* has been described.[159b] The mannan from *T. rubrum* gave lower reactivities than the galactomannan from *A. fumigatus*, against both *S. cerevisiae* and *C. albicans* antisera, since, although more similar in structure to the homologous cell-wall mannan, the short side-chains are incapable of fitting the active sites of both antibodies. The chemical composition of polysaccharides isolated from *C. viswanthii* and *C. tropicalis*[160] and an analysis of the hyphal walls of *Trichopyton mentagrophytes*, *Microsporum canis*, *M. gypseum*, and *Epidermophyton floccosum* have been reported.[160a]

The uronic acid content of the cell-walls of *Mucor rouxii* varied from 25% in the sporangiophore walls to 12% in the hyphal or yeast cell-walls with little or none in the spore-walls.[161] Four distinct polysaccharides were recognised in the sporangiophore-walls,[161a] chitosan, chitin, mucoric acid (largely composed of D-glucuronic acid), and mucoran (an alkali-soluble polymer of D-glucuronic acid, L-fucose, D-mannose, and D-galactose). Glycopeptides containing mannose have been isolated from yeast cell-walls after treatment with ethylene diamine and proteolytic digestion.[162] One glycopeptide, apparently homogeneous, of molecular weight 76,000 contained a high molecular-weight mannan and 4% peptide. Treatment with alkali indicated that one of the carbohydrate-to-peptide linkages is of the *O*-mannosyl serine and threonine type, with only short chains of mannose residues. The bulk of the mannose, in the form of the large molecular weight mannan, was not liberated on treatment with alkali and was probably covalently linked to the peptide by a *N*-(β-aspartyl)-β-D-2-acetamido-2-deoxy-D-glucoside linkage. Mannose-6-phosphate was shown to be an integral part of the mannan structure of the mannose units in the whole glycopeptide, 75–80% were oxidised by periodate with the production of formic acid, and a Smith degradation resulted in the formation of glycerol but no erythritol.

[159a] Y. Fukazawa, N. P. Elinov, T. Shinoda, and T. Tsuchiya, *Jap. J. Microbiol.*, 1968, **12**, 293.
[159b] S. Suzuki, M. Suzuki, K. Yokota, H. Sunayama, and O. Sakaguchi, *Jap. J. Microbiol.*, 1967, **11**, 269.
[160] N. P. Elinov and M. D. Surinova, *Mikrobiologiya*, 1968, **37**, 277.
[160a] V. K. Shah and S. G. Knight, *Arch. Biochem. Biophys.*, 1968, **124**, 229.
[161] S. Bartnicki-Garcia and E. Reyes, *Biochim. Biophys. Acta*, 1968, **170**, 54.
[161a] S. Bartnicki-Garcia and E. Reyes, *Biochim. Biophys. Acta*, 1968, **165**, 32.
[162] R. Sentandreu and D. H. Northcote, *Biochem. J.*, 1968, **109**, 419.

The possibility of the presence of a glycerol-teichoic acid in *Neurospora crassa* has been reported.[163] The production of a capsular polysaccharide by a marine filamentous fungus [163a] and an extracellular polysaccharide by the protoplasts of *Hansenula holstii* [163b] has been studied.

A soil micro-organism has been isolated which will grow on yeast mannans as sole carbon source with accompanying secretion into the medium of an α-mannosidase.[164] This exoglycosidase removed short α-(1 → 2)- and α-(1 → 3)-linked mannose side-chains to leave a core of linear α-(1 → 6)-linked mannose polymer. The mannosidase hydrolysed 6-*O*-α-D-mannopyranosyl-D-mannose and the α-(1 → 2)- and α-(1 → 3)-linked oligosaccharides produced from the yeast mannan by acetolysis. A particulate preparation from *Saccharomyces carlsbergensis* protoplasts incorporated the radioactivity from guanosine diphosphate [^{14}C]mannose into a mannose polymer.[165] The reaction proceeded in the absence of an added primer. The polysaccharide was identified as a yeast mannan by structural investigations. A study of the mode of action on mannans, oligomannans, and reduced oligomannosides led to the conclusion that the β-mannosidase of fenugreek seeds was an endo-β-(1 → 4)-mannanase.[166] An *N*,*O*-diacetyl muramidase from the fungus *Chalaropsis* has been purified and crystallised.[167]

The culture filtrate of the fungus *Coniophora cerebella* grown on a poplar 4-*O*-methyl glucuronoxylan as carbon source and enzyme inducer gave an enzyme system which was able to degrade the polysaccharide to xylose, acidic and neutral oligosaccharides, and an enzyme resistant polymer.[168] Ethanol fractionation led to the isolation of a xylanase (E.C. 3.2.1.8, β-1, 4-xylan xylanohydrolase) and a β-xylosidase (E.C. 3.2.1.37, β-D-xyloside xylohydrolase). The action of the enzyme system on the polysaccharide was entirely random hydrolysis to oligoxylans (DP 2–5) and acidic oligomers of higher molecular weight. The uronic acid residues attached to the polysaccharide protected some of the xylose inter-unit linkages from degradation.

Some properties of the xylanases [169] and cellulases [169a] of *Trichoderma viride*, and the mannosidases [170] and endopolyglycosidases [171] of *Aspergillus*

[163] I. A. Krasheninnikov, I. S. Kulaev, and A. N. Belozerskii, *Doklody Akad. Nauk. S.S.S.R.*, 1967, **172**, 973.
[163a] P. J. Szaniszlo, C. Witzen jun., and R. Mitchell, *J. Bacteriol.*, 1968, **96**, 1474.
[163b] L. P. Kozak and R. K. Bretthauer, *Arch. Biochem. Biophys.*, 1968, **126**, 764.
[164] G. H. Jones and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 2442.
[165] N. H. Behrens and E. Cabib, *J. Biol. Chem.*, 1968, **243**, 502.
[166] S. Clermont-Beaugiraud and F. Percheron, *Bull. Soc. Chim. biol.*, 1968, **50**, 633.
[167] J. H. Hash and M. V. Rothlauf, *J. Biol. Chem.*, 1967, **242**, 5586.
[168] N. J. King and D. B. Fuller, *Biochem. J.*, 1968, **108**, 571.
[169] K. Nomura, T. Yasui, S. Kiyooka, and T. Kobayashi, *J. Ferment. Technol.*, 1968, **46**, 634.
[169a] Y. Tomita, H. Suzuki, and K. Nisizawa, *J. Ferment. Technol.*, 1968, **46**, 701; O. Igarashi, M. Noguchi, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 272.
[170] K. E. Erikson and M. Winell, *Acta Chem. Scand.*, 1968, **22**, 1924.
[171] A. A. Kuznetsov and B. N. Stepanenko, *Biokhimiya*, 1967, **32**, 461.

species have been described. The extent of binding and rate of degradation of α-D-(1 → 3)-linked oligoglucans by an exo-β-D-(1 → 3)-glucanase of *Basidiomycete* species QM806 have been studied.[172]

A model system for the synthesis of alkali-insoluble cell-wall glycogen during differentiation in the slime mould *Dictyostelium discoideum* has been investigated.[173]

[172] F. I. Houtari, J. E. Nelson, F. Smith, and S. Kirkwood, *J. Biol. Chem.*, 1968, **243**, 952.
[173] B. E. Wright, D. Dahlberg, and C. Ward, *Arch. Biochem. Biophys.*, 1968, **124**, 380.

5
Glycoproteins and Glycopeptides

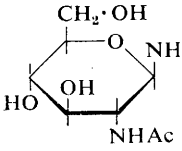
Isolation and General Structural Methods

Xylose found in preparations of pituitary glycoproteins was shown [174] to be an artifact resulting from chromatography on cellulose ion-exchangers, and earlier work which had indicated the presence of glycosidic–ester linkages in ovine and bovine submaxillary gland glycoproteins was shown to be incorrect.[175] The observation that white blood-cells catalysed the conversion of 2-amino-2-deoxy-D-glucose to D-glucose emphasised the importance of determining the products of 2-amino-2-deoxy-D-glucose metabolism in new tissues before using that sugar to label specifically the amino-sugar residues of glycoproteins.[176]

Methods have been described [177] for the fractionation of glycoproteins on hydroxylapatite and agarose gels and for the determination of the molecular weight of glycopeptides by exclusion chromatography.[178] The principle of radioisotope dilution has been applied on a semi-micro scale to the estimation of fucose, mannose, galactose,[179] 2-amino-2-deoxy-glucose, and 2-amino-2-deoxygalactose [180] in some glycoproteins. Values for the non-amino-sugar contents of egg albumin, rabbit γ-globulin and some samples of blood-group substances were similar to the most reliable estimates previously published.

Various groups of workers have studied the enzymic and chemical degradation of carbohydrate–peptide linkages in glycopeptides. A relatively stable *O*-seryl-*N*-acetylgalactosaminide glycohydrolase, free from measurable β-acetylglucosaminidase and proteolytic activities, was purified from homogenates of *Lumbricus terrestris*,[181] and an enzyme capable of catalysing the hydrolysis of 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine (40a) was found in various mammalian organs.[182] A microchemical method for detecting this type of carbohydrate–peptide

[174] J. G. Pierce and T.-H. Liao, *Analyt. Biochem.*, 1968, **24**, 448.
[175] A. Gottschalk and W. König, *Biochim. Biophys. Acta*, 1968, **158**, 358.
[176] S. Kornfield and W. Gregory, *Biochim. Biophys. Acta*, 1968, **158**, 468.
[177] T. Ericson, *Arkiv Kemi*, 1968, **29**, 75.
[178] T. Bhatti and J. R. Clamp, *Biochim. Biophys. Acta*, 1968, **170**, 206.
[179] E. R. B. Graham and A. Neuberger, *Biochem. J.*, 1968, **106**, 593.
[180] E. R. B. Graham and A. Neuberger, *Biochem. J.*, 1968, **106**, 645.
[181] H. Schauer and A. Gottschalk, *Biochim. Biophys. Acta*, 1968, **156**, 304.
[182] T. Ohgushi and I. Yamashina, *Biochim. Biophys. Acta*, 1968, **156**, 417.



R = β-L-aspartyl

(40a)

linkage in glycoproteins has been described [183] (see p. 267). Rat liver lysosomes extensively degraded both the carbohydrate and peptide portions of glycoproteins.[184]

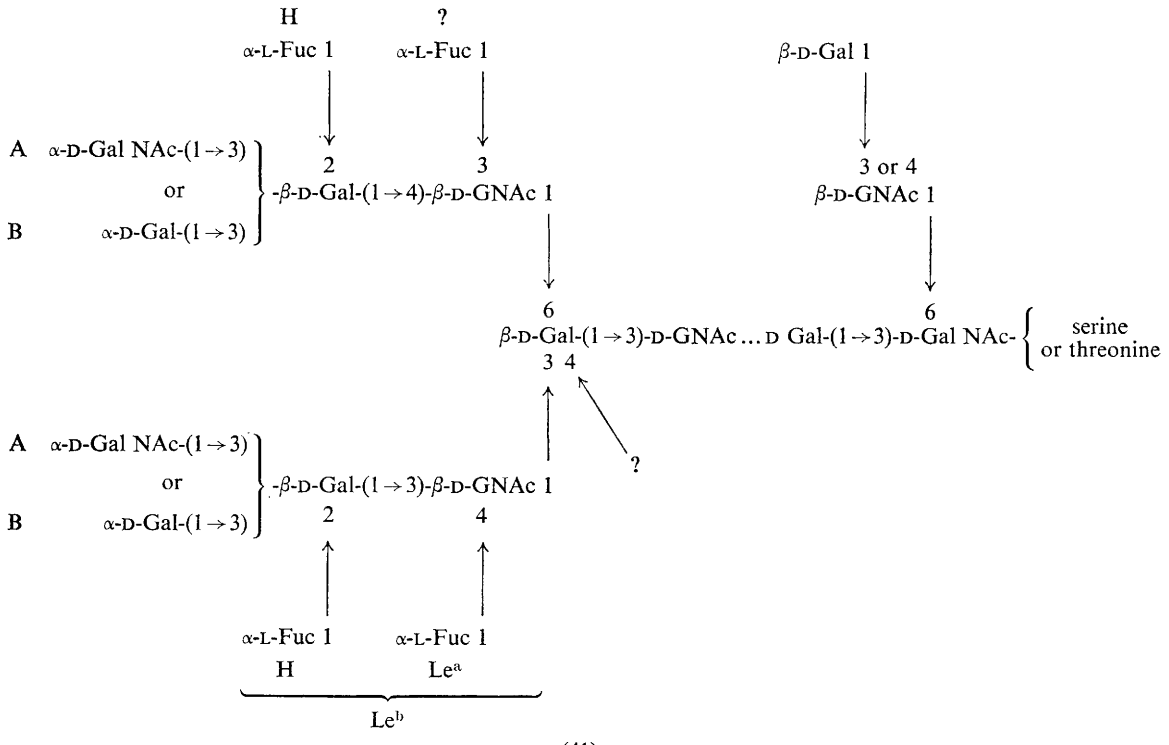
Two-dimensional t.l.c. has been used [185] for comparative studies of oligosaccharides produced by mild hydrolysis of glycoproteins with IR 120 resin (H^+ form) at 100° for 1 hr.

Structural Studies

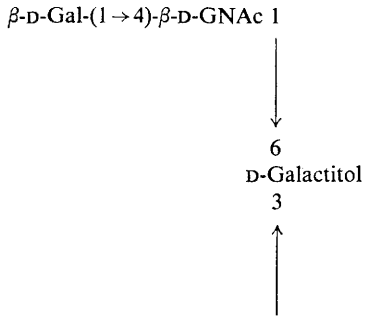
Blood-group Substances.—As a result of further chemical and immunochemical studies by Kabat and co-workers, and by Morgan and co-workers, coupled with evidence from earlier investigations (mainly by these research groups), it was possible to propose an overall structure (41) of the carbohydrate portion of human blood-group A, B, H, Le^a, and Le^b substances, and its mode of attachment to peptide. Much of the evidence for this structure was provided by an extensive investigation by Kabat's group [186] of the structure and immunochemical activities of reduced oligosaccharides (42–47) produced on treatment of blood-group Le^a substance with sodium borodeuteride–sodium deuteroxide. Degradation in this way gave a much simpler pattern of oligosaccharides than was obtained from A, B, and H substances by the same technique.

The mechanism proposed to account for the production of the reduced oligosaccharides involved the initial cleavage of many of the alkali-labile *O*-glycosidic linkages between 2-acetamido-2-deoxy-D-galactose and serine or threonine. The oligosaccharides thus liberated either were reduced with sodium borodeuteride to alkali-stable derivatives or underwent elimination ('peeling' reaction) from the sugar at the reducing end of the oligosaccharide chain to expose a new reducing sugar. The latter was then either reduced or underwent further elimination. The 'peeling' reaction predominantly terminated at certain alkali-stable linkages, *e.g.* to C-6 and C-2 of D-galactose, so that the majority of the reduced oligosaccharides (42–47) had, at what was their reducing ends, products of the alkaline degradation of galactose (mainly hexenetetrols and hexanepentols) or galactitol (see Scheme 1). Direct proof for the occurrence of branch points in the carbohydrate chain was provided by the isolation of oligosaccharide (42).

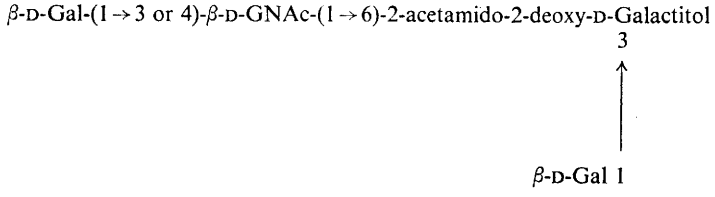
[183] T. H. Plummer jun., A. Tarentino, and F. Maley, *J. Biol. Chem.*, 1968, **243**, 5158.
[184] N. N. Aronson jun. and C. de Duve, *J. Biol. Chem.*, 1968, **243**, 4564.
[185] E. Moczar and M. Moczar, *Bull. Soc. Chim. biol.*, 1967, **49**, 1159.
[186] K. O. Lloyd, E. A. Kabat, and E. Licero, *Biochemistry*, 1968, **7**, 2976.


H
α-L-Fuc 1
?
α-L-Fuc 1
β-D-Gal 1
A α-D-Gal NAc-(1→3)
or
B α-D-Gal-(1→3)
2
3
-β-D-Gal-(1→4)-β-D-GNAc 1
3 or 4
β-D-GNAc 1
6
β-D-Gal-(1→3)-D-GNAc ... D Gal-(1→3)-D-Gal NAc-
6
serine
or threonine
3 4
?
A α-D-Gal NAc-(1→3)
or
B α-D-Gal-(1→3)
-β-D-Gal-(1→3)-β-D-GNAc 1
2
4
α-L-Fuc 1
H
α-L-Fuc 1
Le^a
Le^b

(41)

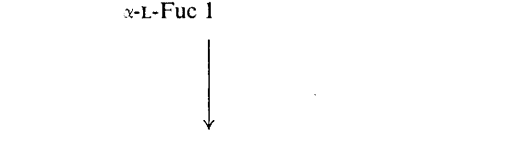


(42)



(43)

(44)



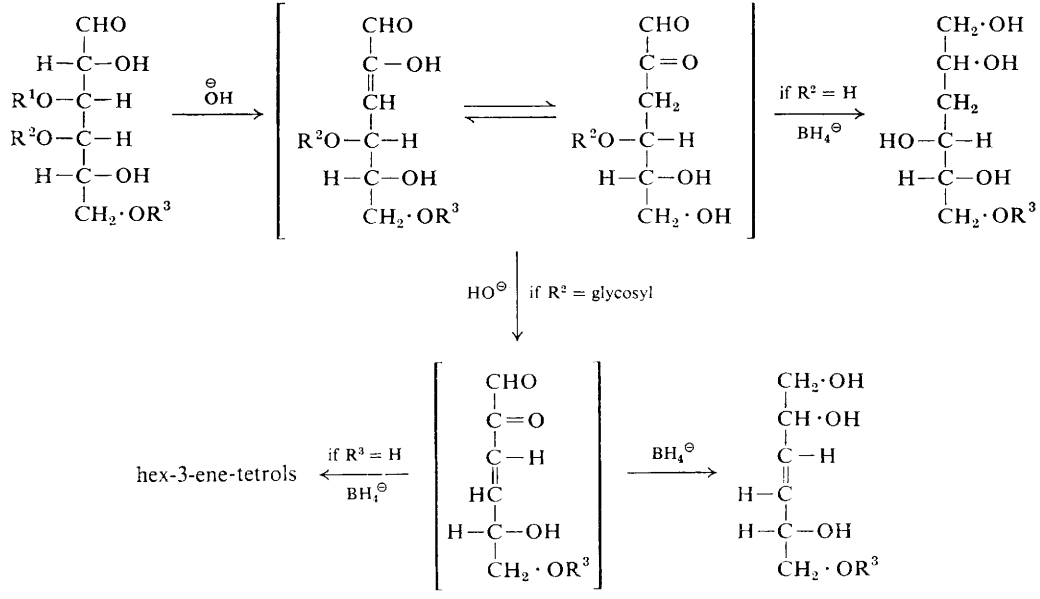
(45)

β-D-Gal-(1→4)-β-D-GNAc-(1→6)-hexane-1,2,4,5,6-pentol(s)

(46)

β-D-Gal-(1→4)-β-D-GNAc-(1→6?)-hex-3-ene-tetrol(s)

(47)



R^1 = β-D-Gal-(1→3)-D-GNAc (and related structures)
R^2 = ?
R^3 = β-D-Gal-(1→4)-D-GNAc (and related structures)

Scheme 1 Mechanism for the formation of unsaturated and deoxy-alcohol residues during the degradation of blood-group substances by sodium borodeuteride–sodium deuteroxide

The proposed composite structure (41) of the blood-group substances represented that of the majority of carbohydrate chains. Evidence was obtained, however, for the presence of other chains including incomplete chains and unbranched chains. Thus oligosaccharide (48) was isolated from A, H, and Le^a substances, (49–51) from A substance, and (44) from

β-D-Gal-(1 → 4)-D-GNAc-(1 → 6)-hexene-tetrol (48)

α-L-Fuc-(1 → 2)-D-Galactitol (49)

α-L-Fuc 1
↓
2
α-D-Gal NAc-(1 → 3)-β-D-Gal-(1 → 3)-β-D-GNAc-(1 → 3 or 4)-D-Galactitol
(50)

α-L-Fuc 1
↓
2
α-D-Gal NAc-(1 → 3)-β-D-Gal-(1 → 4)-β-D-GNAc-(1 → 2 or 6)-D-Galactitol
(51)

Le^a substance. The length of the chains bearing the determinants of blood-group specificity were not determined, but they might be no longer than the length shown in (41).

Studies of the o.r.d. and c.d. spectra of oligosaccharides from blood-group Le^a substance supported the above structural assignments.[187]

Other studies of the structure and biosynthesis of blood-group specific glycoproteins described below confirm the essential correctness of the structure proposed by Kabat's group. Certain of the information is, however, additional to that contained in (41).

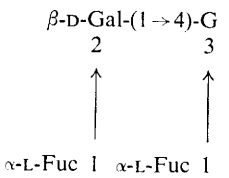
Two new oligosaccharides obtained by alkaline degradation of an Le^a active glycoprotein from ovarian cyst fluid were identified as the tetrasaccharide *O*-β-D-galactosyl-(1 → 4)-[*O*-L-fucosyl-(1 → 3)]-*O*-β-(2-acetamido-2-deoxy-D-glucosyl)-(1 → 3)-D-galactose and a trisaccharide *O*-β-D-galactosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-D-glucosyl)-(1 → 6)-2-acetamido-2-deoxy-D-galactose.[188] A third product was tentatively identified as *O*-β-D-galactosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucosyl-R, where R was an Ehrlich chromogen derived from a 2-acetamido-2-deoxyhexosyl residue.

[187] K. O. Lloyd, S. Beychok, and E. A. Kabat, *Biochemistry*, 1968, **7**, 3762.
[188] A. M. S. Marr, A. S. R. Donald, and W. T. J. Morgan, *Biochem. J.*, 1968, **110**, 789.

The trisaccharide and the chromogen-containing oligosaccharide both inhibited the precipitin reaction between Le^a substance and Type XIV anti-pneumococcal serum.

Trisaccharides of the type D-galactosyl-(2-acetamido-2-deoxy-D-glucosyl)-D-galactose occur in the carbohydrate chains of human blood-group substances. Those oligosaccharides with the (1 → 3)-(1 → 3) and (1 → 4)-(1 → 3) sequences of glycosidic linkages have been described, and two further trisaccharides, with (1 → 3)-(1 → 4)[189] and (1 → 4)-(1 → 6)[190] sequences have been isolated from blood-group H substance by mild hydrolysis with acid. Other oligosaccharides from H substance have been (partially) characterised as *O*-β-D-galactosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-D-glucosyl)-(1 → 6)-*O*-β-D-galactosyl-(1 → 3)-(2-acetamido-2-deoxy-D-glucose);[190] *O*-β-D-galactosyl-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1 → 4)-[*O*-L-fucosyl-(1 → 6)]-D-galactose[191] and *O*-β-D-galactosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-D-glucosyl)-(1 → 6)-*O*-β-D-galactosyl-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1 → 3)-*O*-β-D-galactosyl-(1 → 3)-(2-acetamido-2-deoxy-D-glucose).[191]

A urinary oligosaccharide characteristic of blood-group *O*(H)-secretors was tentatively identified as lactodifucotetraose (52) on the basis of its chemical composition and paper chromatographic mobility.[192]



(52)

The disaccharide 6-*O*-β-(2-acetamido-2-deoxy-D-glucosyl)-D-galactose was obtained on hydrazinolysis of human blood-group A substance.[190] A substance with blood-group A specificity, which was purified from hog gastric mucin by precipitation with the phytohaemagglutinin from *Vicia cracca*, had a different amino-acid composition from that reported by other workers for group A substance from the same source.[193] Thus threonine, serine, and proline together accounted for 85% of the amino-acids by comparison with a typical value of 54% taken from previous publications.

A 2-acetamido-2-deoxy-α-D-galactosyl transferase was found in preparations from human submaxillary glands from group A and AB donors but

[189] W. P. Aston, A. S. R. Donald, and W. M. Watkins, *Biochem. J.*, 1968, **107**, 861.
[190] W. P. Aston, A. S. R. Donald, and W. T. J. Morgan, *Biochem. Biophys. Res. Comm.*, 1968, **30**, 1.
[191] W. P. Aston, A. S. R. Donald, and W. T. J. Morgan, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 508.
[192] A. Lundblad, *Biochim. Biophys. Acta*, 1968, **165**, 202.
[193] T. Kristiansen and J. Porath, *Biochim. Biophys. Acta*, 1968, **158**, 351.

not from group O and B donors.[194] The enzyme fulfilled many of the requirements postulated for the enzymic product of the blood-group A gene. A similar enzyme was found in milk from women of blood types A or AB but was absent in milk from women of groups B or O.[194a]

A serologically active tetrasaccharide, *O*-α-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1 → 3)-D-galactose, was identified as a non-reducing end fragment that was liberated on hydrolysis of human blood-group B active glycoprotein.[195] An α-D-galactosyl transferase associated with the blood-group B character, which was isolated from rabbit stomach mucosal linings, occurred only in the tissues of group B donors [196] and catalysed the following reaction in the formation of the B-specific structure:

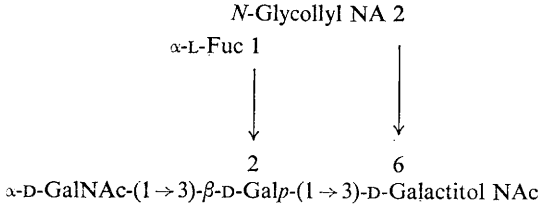
$$\begin{array}{l} \text{UDP-D-Gal} + \beta\text{-D-Gal-R} \\ \qquad\qquad\quad 2 \\ \qquad\qquad\quad \uparrow \\ \qquad\quad \alpha\text{-L-Fuc } 1 \end{array} \xrightarrow{\text{enzyme}} \begin{array}{l} \alpha\text{-D-Gal-}(1 \rightarrow 3)\text{-}\beta\text{-D-Gal-R} \\ \qquad\qquad\quad 2 \\ \qquad\qquad\quad \uparrow \\ \qquad\quad \alpha\text{-L-Fuc } 1 \end{array}$$

where R is the rest of the molecule.

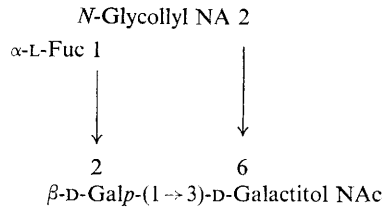
A D-galactosyl transferase which occurred in milk from blood-group B or AB donors, but was absent in milk from blood-group A or O donors, catalysed the transfer of D-galactose from UDP-D-galactose to 2′-fucosyllactose and lacto-*N*-fucopentaose I.[197]

Submaxillary Gland.—Oligosaccharides related to blood-group A substance (see p. 259) were isolated from pig submaxillary mucins following treatment with alkali and borohydride.[198, 199] Aqueous extracts of submaxillary glands from certain pigs contain a mucin (A^+ porcine submaxillary mucin; A^+ PSM) which inhibited the haemagglutination of human type A erythrocytes whereas the mucin (A^- porcine submaxillary mucin; A^- PSM) in aqueous extracts of glands from other pigs lacked this activity.[198] Both mucins, however, contained the sugars, D-galactose, L-fucose, 2-acetamido-2-deoxy-D-galactose, and *N*-glycollylneuraminic acid. Carlson [198] obtained an almost quantitative release of carbohydrate from protein on treatment of the mucins with alkali and borohydride with the production of 2-acetamido-2-deoxy-D-galactitol and a series of reduced oligosaccharides (53–57) which was characterised by acidic and enzymic hydrolyses, methylation studies, periodate oxidation, and Smith degradation. Oligosaccharides (53) and (56), found only in the product mixture from A^+ PSM, were

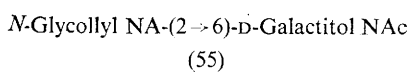
[194] V. M. Hearn, Z. G. Smith, and W. M. Watkins, *Biochem. J.*, 1968, **109**, 315.
[194a] A. Kobata, E. F. Grollman, and V. Ginsberg, *Arch. Biochem. Biophys.*, 1968, **124**, 609.
[195] W. P. Aston, G. M. Hague, A. S. R. Donald, and W. T. J. Morgan, *Biochem. J.*, 1968, **110**, 157.
[196] C. Race, D. Ziderman, and W. M. Watkins, *Biochem. J.*, 1968, **107**, 733.
[197] A. Kobata, E. F. Grollman, and V. Ginsberg, *Biochem. Biophys. Res. Comm.*, 1968, **32**, 272.
[198] D. M. Carlson, *J. Biol. Chem.*, 1968, **243**, 616.


N-Glycollyl NA 2
α-L-Fuc 1
2
6
α-D-GalNAc-(1→3)-β-D-Galp-(1→3)-D-Galactitol NAc

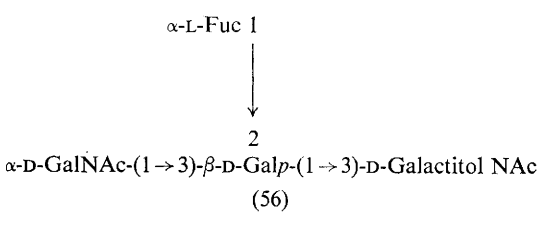
(53)


N-Glycollyl NA 2
α-L-Fuc 1
2
6
β-D-Galp-(1→3)-D-Galactitol NAc

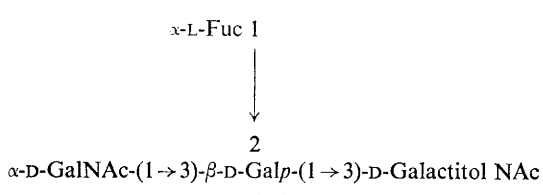
(54)


N-Glycollyl NA-(2→6)-D-Galactitol NAc

(55)


α-L-Fuc 1
2
α-D-GalNAc-(1→3)-β-D-Galp-(1→3)-D-Galactitol NAc

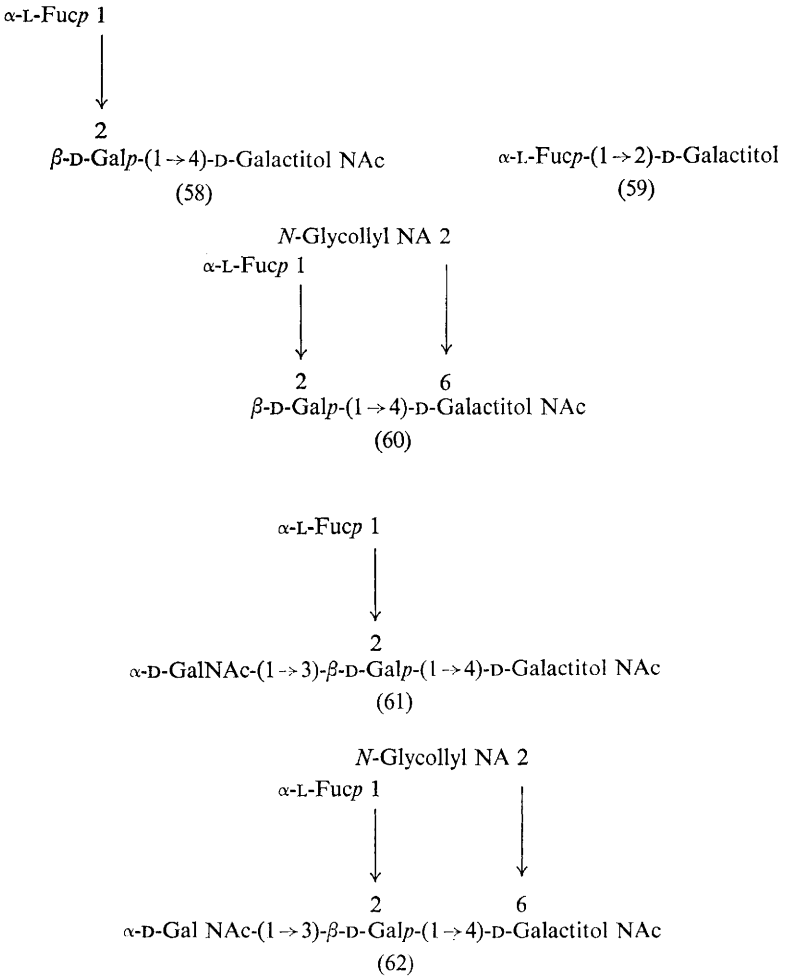
(56)


α-L-Fuc 1
2
α-D-GalNAc-(1→3)-β-D-Galp-(1→3)-D-Galactitol NAc

(57)

potent inhibitors of the precipitin reaction between A^+ PSM and rabbit antiserum to human type A erythrocytes, but oligosaccharides (54), (55), and (57) and 2-acetamido-2-deoxy-D-galactitol were completely inactive. The blood-group A activity of (53) increased following the removal of the sialic acid residue.

In an essentially similar study of pig submaxillary gland by Katzman and Eylar,[199] approximately one-third of the carbohydrate was released, on treatment with alkali and borohydride, in the form of reduced oligosaccharides (58–62), galactitol, and 2-acetamido-2-deoxy-D-galactitol.



[199] R. L. Katzman and E. H. Eylar, *Arch. Biochem. Biophys.*, 1968, **127**, 323.

Characterisation of the oligosaccharides (55, 58–60) by periodate oxidation, Smith degradation, and hydrolysis, and, in some cases, by methylation, showed one essential difference from the oligosaccharides isolated by Carlson, namely, the presence of (1 → 4)- rather than (1 → 3)-linked 2-acetamido-2-deoxy-D-galactitol residues. Reference to (41) (p. 259) shows that both series of oligosaccharides are related to the structure of the carbohydrate part of blood-group A glycoprotein. It is surprising, however, that mixtures of oligosaccharides which contained both types of linkage to 2-acetamido-2-deoxy-D-galactitol were not observed.

Alkaline reductive cleavage of ovine submaxillary mucin liberated 80% of the sialic acid, of which 76% was recovered as *O*-(*N*-acetylneuraminyl)-(2 → 6)-2-acetamido-2-deoxy-D-galactitol, analogous to the *N*-glycollyneuraminyl compound derived from pig submaxillary glycoprotein under similar conditions.[200]

A specific polypeptidyl, 2-acetamido-2-deoxy-D-galactosyl transferase from bovine submaxillary glands, was shown to catalyse the transfer of 2-acetamido-2-deoxy-D-[^{14}C]-galactose to the hydroxy-amino-acid of submaxillary gland glycoprotein.[201] The submaxillary gland glycoprotein, from which sialic acid and 2-acetamido-2-deoxy-hexose had been removed, was the only receptor in the described reaction.

Enzymes.—A micromethod was developed [183] for detecting the 2-acetamido-1-[*N*-β-L-asparty]amino]-2-deoxy-β-D-glucosylamine (40a) linkage in glycoproteins by use of the fluorescent 1-dimethylaminonaphthalene-5-sulphonyl (dansyl) group. The dansyl derivative of (40a) was present in acid hydrolysates of dansylated ribonuclease B glycopeptide and ovalbumin, and the nature of the carbohydrate–peptide linkage was confirmed by hydrolysis with glycopeptide amido-hydrolase from hen oviduct. The polysaccharide thus released from the glycopeptide contained a terminal, reducing residue of 2-acetamido-2-deoxyglucose and a second such sugar residue linked through C-3 or C-4 located internally in the polysaccharide. Minor ribonuclease components of bovine pancreatic juice, designated ribonucleases C and D, both had amino-acid compositions, specific activities, and u.v. spectra identical with those of ribonucleases A and B.[202] Ribonucleases C and D contained 2-amino-2-deoxyglucose (four residues per molecule) and *N*-acetylneuraminic acid (two and four residues per molecule, respectively) and each contained mannose, galactose, and fucose. Evidence of further structural similarities with ribonuclease B was obtained from peptide maps of tryptic digests of the reduced, cyanoethylated enzymes.

α-D-(1 → 4)-D-Glucan glucohydrolase from *Aspergillus niger* was shown [203] to be a glycopeptide [D-mannose (8–15%), D-glucose (2–3%), and D-galactose (0·2%)] in which D-mannose was linked *O*-glycosidically to serine

[200] V. L. N. Murty and M. I. Horowitz, *Carbohydrate Res.*, 1968, **6**, 266.
[201] A. Hagopian and E. H. Eylar, *Arch. Biochem. Biophys.*, 1968, **128**, 422.
[202] T. H. Plummer jun., *J. Biol. Chem.*, 1968, **243**, 5961.
[203] D. R. Lineback, *Carbohydrate Res.*, 1968, **7**, 106.

and threonine. A similar type of carbohydrate–peptide linkage was present in glucamylase II from the same source.

Collagen.—Earthworm cuticle collagen contained 12% of D-galactose and, on treatment with alkali and borohydride, gave reduced di- and tri-saccharides of D-galactose.[204] The oligosaccharides, which accounted for *ca.* 70% of the total carbohydrate, were probably linked to threonine and serine in the collagen molecule. O.r.d. and n.m.r. studies established the presence of α-D-galactosyl linkages within the carbohydrate chains.

A D-glucosyl-D-galactose disaccharide in which C-1 of D-galactose was linked to the δ-hydroxy-group of hydroxylysine was shown to be present in soluble and insoluble collagen.[205] Such glycopeptides therefore were probably not involved in intermolecular cross-links during collagen maturation. A second, heterogeneous, glycopeptide fraction, exclusive to insoluble collagen, contained aspartic and glutamic acids, glycine, alanine, proline, glucose, galactose, mannose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and sialic acid. It was not established, however, whether interpeptide cross-links were present. Evidence was provided for the presence of a limited number of monosaccharide (galactose) side-chains in both forms of collagen.

Galactose and glucose were the only sugars detected in purified preparations of bovine tropocollagen (galactose : glucose, 7 : 3 residues respectively, per 3000 amino-acid residues) and polymeric collagen (4 : 2 residues, respectively, per 3000 amino-acid residues).[206] Digestion of bovine corneal collagen, which contained 2-amino-2-deoxyglucose (0·47%), 2-amino-2-deoxy-galactose (3·08%), fucose (0·02%), mannose (0·21%), xylose (0·31%), glucose (0·57%), and galactose (1·09%), with collagenase and pronase gave two distinct glycopeptides with differing carbohydrate compositions.[207]

Two enzymes, a collagen:galactosyl transferase and a collagen:glucosyl transferase, which together account for the synthesis of the carbohydrate units in collagen, have been found in embryonic guinea-pig skin and the cartilaginous ends of limb bone rudiments.[207a] A collagen:glucosyl transferase was also identified in HeLa cell membranes.[207b]

Fibrin and Fibrinogen.—Four major glycopeptides were isolated from bovine fibrin and fibrinogen; two were characterised by the presence of lysine and the other two by the presence of arginine, glutamic acid, glycine, and valine. One of the lysine-containing glycopeptides, present in both fibrin and

[204] Y. C. Lee and D. Lang, *J. Biol. Chem.*, 1968, **243**, 677.
[205] L. W. Cunningham and J. D. Ford, *J. Biol. Chem.*, 1968, **243**, 2390.
[206] M. E. Grant and D. S. Jackson, *Biochem. J.*, 1968, **108**, 587.
[207] H. B. Bosmann and J. J. Jackson, *Biochim. Biophys. Acta*, 1968, **170**, 6.
[207a] H. B. Bosmann and E. H. Eylar, *Biochem. Biophys. Res. Comm.*, 1968, **30**, 39; 1968, **33**, 340.
[207b] A. Hagopian, H. B. Bosmann, and E. H. Eylar, *Arch. Biochem. Biophys.*, 1968, **128**, 387.

fibrinogen, contained 1·4–2·0 moles of sialic acid.[208] Enzymic degradation of one of the glycopeptides indicated the non-reducing sequence as sialic acid → galactose → 2-acetamido-2-deoxy-glucose → mannose. *N*-Acetyl and *N*-glycollyl neuraminic acid were present in all the glycopeptides. Further structural information was obtained[209] by characterisation of oligosaccharides (63–66) liberated on partial hydrolysis with acid of fibrinoglycopeptides.

Sialic acid-(2→6)-β-Gal-(1→4)-GNAc (63)
Sialic acid-(2→3)-β-Gal-(1→4)-GNAc (64)
β-Gal-(1→4)-GNAc (65)
Man-(1→6)-Man (66)

Evidence was obtained for the presence of a carbohydrate–peptide linkage between the β-carboxy-group of aspartic acid and the amino-group of 2-acetamido-2-deoxy-β-D-glucopyranosylamine.

Serum Glycoproteins.—*α_1- and α_2-Acid Glycoproteins.* The α_2-macroglobulin of human plasma was shown to contain galactose, 1·36%, 2-acetamido-2-deoxyglucose, 3·94%; mannose, 2·07%, *N*-acetylneuraminic acid, 1·80%; and fucose, 0·26%; and all the amino-acids common to globular proteins.[210] For a molecule of molecular weight 820,000 it was concluded that there were approximately 363 monosaccharide residues and 6463 amino-acid residues. Subsequent structural studies of glycopeptides obtained from α_2-macroglobulin by digestion with pronase indicated the presence of 31 carbohydrate units per molecule of α_2, with an average composition of mannose (3), galactose (2), 2-acetamido-2-deoxyglucose (4·7), *N*-acetylneuraminic acid (1·5), and fucose (0·4), most, or all, of which were joined to peptide by a glycosylamine-type linkage involving asparagine.[211] The smallest carbohydrate unit, proposed as an internal sequence of the carbohydrate portion, contained mannose (3) and 2-amino-2-deoxyglucose (2). Others contained, in addition, variable amounts of sialic acid, fucose, and galactose, and additional 2-amino-2-deoxyglucose. The sequence sialic acid (or fucose) → galactose → 2-acetamido-2-deoxyglucose was determined and the variations in the carbohydrate unit appeared to be primarily a function of the number and degree of completion of these oligosaccharides present in each carbohydrate unit. The largest carbohydrate unit in the α_2-globulin macromolecule was highly branched and was thought to contain as many as four such chains linked to the core of mannose and 2-acetamido-2-deoxyglucose residues. 4-*O*-β-D-Galactopyranosyl-2-acetamido-2-deoxyglucose and mannobiose were shown to be sequences in the carbohydrate portion. The ability of horse serum to specifically

[208] B. A. Bray and K. Lakin, *Biochemistry*, 1968, **7**, 3119.
[209] L. Mester, E. Moczar, and L. Szabados, *Compt. rend.*, 1967, **265**, (*C*), 877.
[210] J. T. Dunn and R. G. Spiro, *J. Biol. Chem.*, 1967, **242**, 5549.
[211] J. T. Dunn and R. G. Spiro, *J. Biol. Chem.*, 1967, **242**, 5556.

inhibit the A2 strain of influenza virus was determined by a 4-*O*-acetyl-*N*-acetylneuraminic acid-containing α_2-macroglobulin.[212]

Other workers isolated 6-*O*-mannosylmannose and 3-*O*-mannosylmannose from ovomucoid and α_1-acid glycoprotein by partial hydrolysis with acid.[213] Galactose and mannose were liberated from sialic acid-free α_1-acid glycoprotein by treatment with enzymes from beef liver,[214] and a series of peptides and glycopeptides were produced on incubation of the carboxymethyl-derivative of α_1-acid glycoprotein with trypsin.[215]

Gel electrophoretic studies showed that the relative concentration of α_1-acid glycoprotein in serum was depressed during pregnancy.[216]

Immunoglobulins. A Cold Spring Harbor Symposium on 'Antibodies' included sections on the structure of antibodies, evolution and genetics of antibodies, synthesis of antibodies, and differentiation and cellular events.[217]

It has been shown that the oligosaccharide units of an I_gA type K myeloma globulin contribute to the antigenicity of the molecule.[218] Some I_gA molecules were hydrolysed by neuraminidase with appropriate alteration in the electrophoretic mobility, whereas in others the sialic acid resisted hydrolysis.[219] Such sialic acid residues were hydrolysed, however, after preliminary proteolysis.

Detailed structural studies were reported of twelve glycopeptides isolated after digestion of I_gA myeloma globulin with pronase.[220] The intact A myeloma globulin contained L-fucose (2), D-mannose (14–15), D-galactose (12–13), 2-acetamido-2-deoxy-D-glucose (12–13), 2-acetamido-2-deoxy-D-galactose (6), and *N*-acetylneuraminic acid (5) residues distributed between six oligosaccharide units, all of which were present in the heavy polypeptide chains. Examples of so-called central and peripheral heterogeneity were found in the glycopeptides in that the carbohydrate portion of some had totally different structures (central heterogeneity) whereas others had the same core structure but small differences in terminal residues (peripheral heterogeneity). Three types of core oligosaccharide units were identified. Type 1 included glycopeptide 1: NANA → 3 Gal → 6GalNAc → 3 or 4 GalNAc → 2 or 4 GalNAc → Ser and glycopeptide 4 (as glycopeptide 1, without NANA); Type 2 (glycopeptides 2, 5–12) had a fundamental structure comprising D-mannose, D-galactose, and 2-acetamido-2-deoxy-D-glucose (3 : 2 : 3) relative to one residue of aspartic acid. Associated with

[212] D. S. Pepper, *Biochim. Biophys. Acta*, 1968, **156**, 317.
[213] B. Fournet, G. Takerkart, J. Brohon, and J. Montreuil, *Bull. Soc. Chim. biol.*, 1968, **50**, 1352.
[214] T. Langley, *Arch. Biochem. Biophys.*, 1968, **128**, 304.
[215] R. Bourrillon and D. Meyer, *Bull. Soc. Chim. biol.*, 1967, **49**, 1127.
[216] J. B. Adams and A. Wacher, *Clinica. Chim. Acta*, 1968, **21**, 155.
[217] Cold Spring Harbor Symposium, 1967, **32**. Waverly Press, Baltimore, Maryland, U.S.A.
[218] J. R. Clamp and J. V. Jones, *Clinica. Chim. Acta*, 1968, **21**, 165.
[219] G. B. Richard, P. Silberzahn, M. Bonnassieux, and L. Colobert, *Clinica. Chim. Acta*, 1968, **21**, 479.
[220] G. Dawson and J. R. Clamp, *Biochem. J.*, 1968, **107**, 341.

this structure were 0, 1, 2, or 3 residues of sialic acid; 1-O residues of L-fucose and 1-O residues of 2-acetamido-2-deoxy-D-glucose. Glycopeptides 7, 10, and 12 contained aspartic acid as the only amino-acid and 1, 2, and 3 moles of sialic acid, respectively. The other Type 2 glycopeptides contained amino-acids in addition to aspartic acid. The following tentative structure was proposed for the Type 2 series of glycopeptides:

Man→2	or	4Man→2	or	4Man→3	or	4GNAc→Asp
2 or 4		2 or 4		2 or 4		3, 4 or 6
↑		↑		↑		↑
GNAc		GNAc		GNAc		Fuc
3 or 4		3 or 4				
↑		↑				
Gal		Gal				
2 or 4		2 or 4				
↑		↑				
NANA		NANA				

Glycopeptides 11 and 12, which contained 3 moles of NANA, had either two NANA residues linked to the same galactose residue, or two NANA residues linked together and linked to galactose; Type 3 (Glycopeptide 3) was assigned the tentative structure: Man→3 Man→3 Man→3 or 4 GNAc→3 or 4 GNAc→3 or 4 GNAc→asp. It was proposed that I_gA myeloma globulin contained, per molecule, an average of two Type 1, three Type 2, and one Type 3 oligosaccharide units.

Structures (67) and (68) were proposed [221] for two glycopeptides isolated from the deoxycholate-soluble fraction of rat liver microsomes 90 min. after the injection of 2-amino-2-deoxy-[1-^{14}C]glucose. The suggested structure

α-Man→α-Man→β-GNAc→ [Man (1) / GNAc (2)] →peptide

↑

Sialic acid→β-Gal→β-GNAc→α-Man→α-Man

(67)

α-Man→α-Man→α-Man→α-Man→β-GNAc→α-Man

↓

[Man (3)]→GNAc–Asp

↑

β-GNAc→α-Man→β-GNAc

(68)

[221] J.-T. Li, S.-C. Li, and M. R. Shetlar, *J. Biol. Chem.*, 1968, **243**, 656.

for (67) was only one of several structures consistent with the available data. The authors proposed that these glycopeptides might carry the sugar units which occupy the inner core of the mature plasma structure.

Studies of the carbohydrate content of fragments and polypeptide chains of human γG myeloma proteins of different heavy-chain subclasses showed [222] that, of 76 proteins, 29% had more than 19 moles of hexose per mole by comparison with 9–13 moles per mole in the remaining 71%. There was no correlation, however, between the hexose content and the antigenic subclass of the heavy- or light-chain type. The excess of carbohydrate on the 29% of the proteins was located on the so-called Fab fragment.

From studies of the glycopeptides produced by digestion of human γ-M immunoglobulin with trypsin it was concluded that the H chains of that immunoglobulin contained 5–6 oligosaccharides.[223] A survey of human pathological macroglobulins showed that γ-M could be divided into two groups on the basis of their carbohydrate composition.[224] The different carbohydrate contents of Group I (10·69 ± 1·49%) and Group II (7·71 ± 0·65%) were attributed to differences in hexose contents. Treatment of the macroglobulins from each group with pronase gave three types of glycopeptide. Glycopeptides 1 from both groups contained mannose, galactose, and 2-acetamido-2-deoxyglucose (3 : 2 : 1 in glycopeptide 1 from Group I; 2 : 1 : 2 in glycopeptide 1 from Group II); glycopeptide 2 from Group I contained mannose, galactose, and 2-acetamido-2-deoxy-glucose (9 : 1 : 2) whereas that from Group II contained mannose, fucose, galactose, and 2-acetamido-2-deoxyglucose (2 : 1 : 3 : 2); glycopeptides 3 from both groups contained mannose, fucose, galactose, 2-acetamido-2-deoxyglucose, and sialic acid (6 : 2·5 : 2·5 : 5·5 : 2 for Group I; 5 : 1 : 1 : 6 : 1 for Group II). It was proposed that γ-M macroglobulin was probably composed of ten glycopeptides 1 and 2 and twenty glycopeptide 3 molecules per molecule.

Urinary Glycoproteins.—Glucosylgalactosylhydroxylysine, in which C-1 of galactose and the hydroxy-group of hydroxylysine were involved in the glycopeptide linkage, has been isolated from the urine of neuroblastoma patients.[225] Comparative studies of the Tamm–Horsfall glycoprotein purified from normal urine and from urine of patients with nephritis and sucrosuria showed [226] that glucose was a structural component and that the level of hexoses (galactose, mannose, and glucose) was increased in samples from the two pathological conditions. Glycopeptides isolated from the urinary protein excreted in heavy-chain disease were separated by ion-exchange chromatography into two broad fractions.[227] Fraction 1

[222] C. A. Abel, H. L. Spiegelberg, and H. M. Grey, *Biochemistry*, 1968, **7**, 1271.
[223] R. Bourrillon and E. Razafimahaleo, *Bull. Soc. Chim. biol.*, 1967, **49**, 1115.
[224] J. M. Davie and C. K. Osterland, *J. Exptl. Med.*, 1968, **128**, 699.
[225] H. Shimizu and E. H. Labrosse, *Clinica. Chim. Acta.*, 1968, **22**, 623.
[226] E. L. Rozenfel'd and N. A. Yusipova, *Biokhimiya*, 1967, **32**, 111.
[227] J. R. Clamp, G. Dawson, and E. C. Franklin, *Biochem. J.*, 1968, **110**, 385.

contained L-fucose (3–4 residues), D-mannose (10), D-galactose (5–6), 2-acetamido-2-deoxyglucose (12), and *N*-acetylneuraminic acid (4–5), based on a dimeric molecular weight of *ca.* 51,000. The corresponding values for Fraction 2, which also contained one residue of 2-acetamido-2-deoxygalactose, were 2, 10, 7, 12, and 7. Fraction 1 contained an average of four oligosaccharide units, two of which contained L-fucose (1), D-mannose (3), D-galactose (1), and 2-acetamido-2-deoxyglucose (3); the other two contained the same proportions of L-fucose, D-mannose, and 2-acetamido-2-deoxyglucose together with D-galactose (2) and *N*-acetylneuraminic acid (2). Fraction 2 also contained an average of four oligosaccharide units but a wider range of glycopeptides containing L-fucose (1–0), D-mannose (2–3), D-galactose (2–3), 2-acetamido-2-deoxyglucose (2–3), and *N*-acetylneuraminic acid (2–3). In addition, glycopeptides containing D-mannose (1), D-galactose (2), 2-acetamido-2-deoxy-galactose (1), and *N*-acetylneuraminic acid (0–3) were present in Fraction 2.

Miscellaneous Glycoproteins.—A glycoprotein containing fucose (3%), galactose (7%), and 2-acetamido-2-deoxyglucose (4%) has been isolated from red cell mucoid [228] and one containing 20–25% carbohydrate (galactose, mannose, fucose, xylose, glucose, 4 : 2 : 1 : 3 : 1; 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, 2 : 3) of which 8–10% was sialic acid, was isolated from erythrocyte stroma.[229] The microheterogeneity observed on starch-gel electrophoresis of fetuin, a fetal calf serum glycoprotein, was attributed [230] primarily to the presence of varying amounts of sialic acid in the fractions obtained on starch-gel electrophoresis. A glycoprotein, purified from cold aqueous alkali extracts of the walls of human aorta, contained *ca.* 8% of carbohydrate, all of which was present in a glycopeptide (molecular weight *ca.* 7800) obtained by pronase digestion of the glycoprotein.[231] The glycopeptide contained sialic acid (in terminal positions on the carbohydrate chains), galactose, mannose, fucose, and 2-amino-2-deoxyhexose (5 : 10 : 5 : 2 : 11).

Three hyaluronidase-resistant sulphated glycoproteins purified from dog gastric mucosa were separated on DEAE-Sephadex by elution with a sodium chloride gradient.[232] Each glycoprotein contained equimolar amounts of 2-amino-2-deoxyhexose and galactose, and equimolar amounts of 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose. The molar ratio of sulphate to 2-amino-2-deoxyhexose varied from 0·11 to 0·45 and the amounts of fucose and sialic acid in the glycoproteins decreased as the concentration of sodium chloride in the DEAE-Sephadex eluate increased. The total protein content varied from 10·9 to 17% and the preponderant amino-acids were threonine, serine, proline, and valine. *N*-Sulphate was

[228] G. Uhlenbeck, H. Hansen, and G. I. Pardoe, *Z. physiol. Chem.*, 1968, **349**, 733.
[229] H. Weicker, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 398.
[230] Y. Oshiro and E. H. Eylar, *Arch. Biochem. Biophys.*, 1968, **127**, 476.
[231] M. J. Barnes and S. M. Partridge, *Biochem. J.*, 1968, **109**, 883.
[232] T. Pamer, G. B. J. Glass, and M. I. Horowitz, *Biochemistry*, 1968, **7**, 3821.

absent and both fucose and sialic acid were liberated by mild hydrolysis with acid. Two glycosaminoglycans isolated from the same source were tentatively identified as dermatan sulphate and heparitin.

In a series of papers Kent, Allen and Draper reported studies of the biosynthesis of sheep intestinal mucins. *N*-Acetylneuraminic acid, *N*-glycollylneuraminic acid, and a diacetylated sialic acid were present in sheep colonic epithelial mucin, and a particle-free enzyme preparation obtained from sheep colonic mucosa was shown to catalyse a series of reactions, involving 2-acylamine-2-deoxysugars, which lead to the formation of the sialic acids *in vitro*.[233] The incorporation of L-threonine and D-glucose into a well-characterised glycosaminoglycan fraction of sheep colonic mucosal tissue was inhibited by puromycin.[234] The inhibitory effect of salicylate on glycoprotein biosynthesis by sheep colonic and human gastric mucosal tissue probably occurred at the level of amino-sugar intermediates.[235] *In vitro* experiments showed[236] that the radioactivity from D-[2-^{14}C]glucose, [2-^{14}C]acetate, [3-^{14}C]pyruvate, and hydroxy-[3-^{14}C]-pyruvate was incorporated solely into all the monosaccharide components of the major glycoprotein component from sheep colonic mucosa, whereas the radioactivity from [*u*-^{14}C]glycine was incorporated into the protein part of the glycoprotein. Scrapings of sheep colonic mucosa utilised glucose and acetate for the biosynthesis of the *N*-acetyl and *N*-glycollyl moieties of sialic acids, but the pathway of *N*-glycollylation was not determined.

Two glycopeptides (molecular weights $2{\cdot}3 \times 10^3$ and $2{\cdot}0 \times 10^3$, respectively) isolated from pronase-digests of the low-density lipoprotein of hen's egg yolk contained mannose (55 and 64%, respectively), 2-amino-2-deoxyhexose (14 and 17%), *N*-acetylneuraminic acid (12 and 0%), and probably occurred in the lipoprotein with the heteropolysaccharide linked *N*-glycosidically *via* the β-amido-group of an asparagine residue.[237] Ten glycopeptides isolated from pronase-digests of hen ovomucoid each contained 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine as the sole carbohydrate–peptide linkage region.[238] 6-*O*-Mannosylmannose and 3-*O*-mannosylmannose have been isolated[213] from ovomucoid by partial hydrolysis with acid. Comparative studies of the glycopeptides obtained on proteolytic digestion of ovotransferrin (conalbumin) and transferrin of the hen showed that, in the former, the bulk of the carbohydrate was present as a single oligosaccharide composed of mannose (4) and 2-amino-2-deoxyglucose (8) attached to an aspartic acid residue, and, in the latter, most of the carbohydrate was present in a single unit composed of

[233] P. W. Kent and P. Draper, *Biochem. J.*, 1968, **106**, 293.
[234] A. Allen and P. W. Kent, *Biochem. J.*, 1968, **106**, 301.
[235] P. W. Kent and A. Allen, *Biochem. J.*, 1968, **106**, 645.
[236] A. Allen and P. W. Kent, *Biochem. J.*, 1968, **107**, 589.
[237] J. Z. Augustyniak and W. G. Martin, *Canad. J. Biochem.*, 1968, **4**, 983.
[238] M. Monsigny, A. Adam-Chosson, and J. Montreuil, *Bull. Soc. Chim. biol.*, 1968, **50**, 857.

mannose (2), galactose (2), 2-amino-2-deoxyglucose (3), and sialic acid (1 or 2).[239] The amino-acid sequences in the glycopeptides which carried these different glycopeptides were the same.

A glycoprotein containing galactose, mannose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, sialic acid, and small amounts of fucose was isolated from tissue-cultured mouse mast-cell tumours.[240] The alkali-stability of the glycoprotein indicated that *O*-glycosylated hydroxy-amino-acids probably were not involved in the carbohydrate–peptide linkage.

Further studies of glycopeptide pituitary hormones have been reported. Work on the isolation of human follicle-stimulating hormone, from the pituitary gland and from postmenopausal urine, and a comparative study of some of the chemical, physical, immunological, and biological properties of this hormone from these two sources, which was originally cited from a Ph.D. dissertation (see Vol. 1, p. 253) has been published.[241] Bovine and ovine interstitial cell-stimulating hormones were reported to contain mannose (4–5 and 7 residues, respectively), galactose (1–2 and 1–2), fucose (1–2 and 2), 2-amino-2-deoxyglucose (5–6 and 8), and 2-amino-2-deoxy-galactose (2–3 and 3) and showed markedly diminished activity after treatment with periodate ion which destroyed fucose, galactose, mannose, and half-cystine residues.[242] ^{14}C-labelled galactose, 2-amino-2-deoxyglucose, proline, and leucine were shown to be incorporated into biologically and immunologically active bovine lutenising hormone.[243]

A sulphated glycoprotein containing fucose (5·9%), mannose (2·2%), glucose (0·8%), galactose (0·8%), 2-amino-2-deoxyhexose (7·9%), sulphate (20%), and 16 amino-acids was released on fertilisation of eggs of the sea-urchin *Hemicentrotus pulcherrimus*.[244]

An enzyme purified from the liver of the marine gastropod *Charonia lampas* catalysed the hydrolysis of *O*-β-xylosyl L-serine and plant glycoprotein stem bromelain with the liberation of xylose.[245] It was concluded that the plant glycopeptide contained non-reducing, terminal xylose.

Enzyme and chemical degradation of a glycopeptide from soy-bean haemagglutinin showed [246] that the carbohydrate chains contained blocks of mannose residues separated by 2-acetamido-2-deoxyglucose residues. Of the mannose units 40% resisted oxidation by periodate and some of the 2-acetamido-2-deoxyglucose units were (1 → 6)-linked.

A mechanism has been proposed [247] for the oxidative gelation of a glycoprotein from wheat flour. Studies of the incorporation of

[239] J. Williams, *Biochem. J.*, 1968, **108**, 57.
[240] D. B. Thomas, *Biochem. J.*, 1968, **109**, 79.
[241] P. Roos, *Acta Endocrinol. Suppl.*, 1968, **131**, 1.
[242] J. Gan, H. Papkoff, and C. H. Li, *Biochim. Biophys. Acta*, 1968, **170**, 189.
[243] M. F. Winnick, *Proc. Natl. Acad. Sci. U.S.A.*, 1968, **59**, 1009.
[244] K. Ishihara, *Exptl. Cell Res.*, 1968, **51**, 473.
[245] M. Fukuda, T. Muramatsu, F. Egami, N. Takahashi, and Y. Yasuda, *Biochim. Biophys. Acta*, 1968, **159**, 215.
[246] H. Lis, *Israel J. Chem.*, 1968, **6**, 114P.
[247] T. J. Painter and H. Neukom, *Biochim. Biophys. Acta*, 1968, **158**, 363.

isotopically-labelled D-glucose and *N*-acetylneuraminic acid by particles from bovine retina indicated a sequential addition of D-galactose and *N*-acetylneuraminic acid to the same carbohydrate chain of an endogenous acceptor.[248] Two glycoprotein:fucosyl transferases present in HeLa cells have been characterised.[249] One enzyme catalysed the transfer of fucose to the terminal, non-reducing 2-acetamido-2-deoxyglucose in fetuin from which sialic acid and galactose had been removed. The other catalysed the formation of *O*-α-L-fucose-(1 → 2)-D-galactose with substrates, such as porcine submaxillary mucin from which sialic acid and fucose had been removed, which contained terminal, non-reducing galactose. A polypeptidyl:2-acetamido-2-deoxygalactosyl transferase, a collagen:fucosyl transferase, and a glycoprotein:galactosyl transferase have been identified in HeLa cell membrane fractions.[207b]

Protein-containing molecules, in which 2-amino-2-deoxy-D-[1-^{14}C]glucose was incorporated, were shown to exist in non-specialised cancerous and normal cells cultivated *in vitro*.[250] The preferential sites of incorporation were located at endoplasmic membranes, and especially at rough membranes.

[248] P. J. O'Brien and C. S. Mullenberg, *Biochim. Biophys. Acta*, 1968, **158**, 189.
[249] H. B. Bosmann, A. Hagopian, and E. H. Eylar, *Arch. Biochem. Biophys.*, 1968, **128**, 470.
[250] R. Got, J. Frot-Coutaz, L. Colobert, and P. Louisot, *Biochim. Biophys. Acta*, 1968, **157**, 599.

6
Acidic Glycosaminoglycans

Polysaccharide Sulphates and Hyaluronic Acid from Animal Tissues

Sulphated glycosaminoglycans have been found in a wide range of animal tissues including mammalian brains,[251] foetal pig epidermis,[252] hog skin,[253] frog skin,[254] normal and cirrhotic livers,[255] mouse foetus and placenta,[256] human leucocytes,[257] tendon,[258] aorta,[259] and bone.[260] Pearce and co-workers[261] investigated the ion-exchange properties of Dowex 1×2 (chloride form) for the common anionic glycosaminoglycans found in animal tissue and concluded that the separations obtained depended to a greater extent on the support and conditions of elution than on the properties of the glycosaminoglycans.

The major polysaccharide extracted from cattle retina with saline has been identified as a 'half-sulphated' chondroitin sulphate.[262] Other carbohydrate-containing components in the extract included water-soluble and water-insoluble heteropolysaccharides which contained 2-amino-2-deoxyglucose, galactose, and sialic acid, and gangliosides.

The changes that occur in acidic glycosaminoglycans of liver in early and advanced stages of chronic hepatic damage have been reported.[262a] Studies of the kinetics of the turnover of ^{35}S-labelled acidic glycosaminoglycans in fibroblasts from skin showed an accumulation of intracellular acidic glycosaminoglycans in Hurler's and Hunter's syndromes.[262b] The kinetics of turnover were converted to near normal, however, if the cells

[251] W. L. Cunningham and J. M. Goldberg, *Biochem. J.*, 1968, **110**, 35P.
[252] J. G. Smith jun. and E. A. Davidson, *Biochim. Biophys. Acta*, 1968, **165**, 182.
[253] J. A. Cifonelli and L. Rodén, *Biochim. Biophys. Acta*, 1968, **165**, 553.
[254] M. J. Lipson and J. E. Silbert, *Biochim. Biophys. Acta*, 1968, **158**, 344.
[255] A. Delbrück, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 460.
[256] J. Švejcar, *Biochim. Biophys. Acta*, 1968, **165**, 84.
[257] M. F. Kharchenko and I. F. Seits, *Biokhimiya*, 1968, **33**, 43.
[258] R. W. Dormer, C. A. Antonopoulos, and S. Gardell, *Biochim. Biophys. Acta*, 1968, **158**, 336.
[259] M. D. Franek and J. R. Dunstone, *Biochim. Biophys. Acta*, 1968, **165**, 555.
[260] G. M. Herring, *Biochem. J.*, 1968, **107**, 41.
[261] R. H. Pearce, J. M. Mathieson, and B. J. Grimmer, *Analyt. Biochem.*, 1968, **24**, 141.
[262] E. R. Berman and G. Bach, *Biochem. J.*, 1968, **108**, 75.
[262a] T. Koizumi, N. Nakamura, and H. Abe, *Biochim. Biophys. Acta*, 1967, **148**, 749.
[262b] J. C. Fratantoni, C. W. Hall, and C. F. Neufeld, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **60**, 699.

from Hunter's or from Hurler's syndrome were incubated with each other, or if cells from either type were incubated with normal cells. These results suggested that the genetic lesion in each abnormal cell line was the lack of a diffusable factor, necessary for the degradation of acidic glycosaminoglycans, which could be supplemented by cells of a different genotype. Other workers[262c] have noted the intracellular accumulation of high molecular weight forms of hyaluronic acid and dermatan sulphate by Hurler fibroblasts.

The components of human urine that were precipitated by cetyltrimethylammonium bromide were moderately increased following therapeutic enzymic dissolution of the nucleus pulposus, and greatly increased after surgical stress.[262d] The latter condition resulted in the excretion of an apparently unique, non-diffusible polyuronide.

Structural Studies

General Chemical and Enzymic Methods.—A simple glycosulphatase produced by the mould *Trichoderma viride* grown on a defined medium containing the potassium salt of either D-glucose 6-sulphate or D-galactose 6-sulphate catalysed the hydrolysis of the 6-sulphates of D-glucose and D-galactose, but not the 3-sulphate of D-glucose.[263] The activity of glycosulphatases against sulphate esters of D-galactose and mixed substrates was determined by estimating the D-glucose and D-galactose produced by means of the enzymes D-glucose oxidase and D-galactose oxidase.[264] In this way the glycosulphatase from *Patella vulgata* was shown to act on the 6-sulphates of both D-glucose and D-galactose. The combined use of enzymic and chemical methods for the characterisation of hyaluronic acid, chondroitin 4- and chondroitin 6-sulphates on a 150 μg scale has been described.[265]

The reduced rates of oxidation by periodate which have been observed for acidic polysaccharides have been attributed to the exclusion of the negatively charged periodate ions from the domain of the acidic polysaccharides.[265a] This electrostatic effect was suppressed by adding salt (0·2M-sodium perchlorate). Since the polymer charge due to weakly acidic groups ($-CO_2H$) was greatly reduced in periodic acid, oxidation in periodic acid (pH <3) was faster than in equivalent concentrations of sodium metaperiodate (pH >5). The rate of oxidation of hyaluronate and heparin, although considerably increased by the addition of sodium

[262c] R. Matalon and A. Dorfman, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **60**, 179.
[262d] I. J. Stern, F. Cosmas, and L. Smith, *Clinica. Chim. Acta*, 1968, **21** 181.
[263] A. G. Lloyd, P. J. Large, M. Davies, A. H. Olaveson, and K. S. Dodgson, *Biochem. J.*, 1968, **108**, 393.
[264] R. J. Fielder and P. F. Lloyd, *Biochem. J.*, 1968, **109**, 14P.
[265] S. A. Barker, J. F. Kennedy, and P. J. Somers, *Carbohydrate Res.*, 1968, **8**, 482.
[265a] J. E. Scott and R. J. Harbinson, *Histochemie*, 1968, **14**, 3215.

perchlorate, was slow or very slow by comparison with that of dextran and polygalacturonate. The glycol group at C-2 and C-3 of the uronic acid portion was shown to be primarily the target for oxidation.

Chondroitin Sulphates.—An inducible chondroitinase has been purified from *Proteus mirabilis*. No chondrosulphatase was detected in crude or purified fractions.[265b] Desulphation of chondroitin 4-sulphate without prior depolymerisation was achieved with an enzyme preparation from *P. vulgata* in the presence of cyanide ion which inhibited the polysaccharase activity also present in the preparation.[266] An enzyme designated 'chondroitinase ABC' has been purified to apparent homogeneity from extracts of *P. vulgaris* NCTC 4636 adapted on a medium containing chondroitin 6-sulphate.[267] It catalysed the degradation of chondroitin 4- and 6-sulphate and dermatan sulphate to 4,5-unsaturated oligosaccharides, but showed no activity against keratan sulphate, heparin, or heparitin sulphate. A 'chondroitinase AC' from *Flavobacterium heparinum* ATCC 13125, adapted on a medium containing chondroitin 6-sulphate, showed no measurable activity against dermatan sulphate, but catalysed essentially the same reaction as 'chondroitinase ABC' with chondroitin 4- and 6-sulphates. Among other enzymes produced by this bacterium was a glucuronidase which catalysed the hydrolysis of the β-glucuronidic bond of the 4,5-unsaturated, but not of the saturated oligosaccharides. Two sulphatases, designated chondro-4-sulphatase and chondro-6-sulphatase, have been isolated from the crude *Proteus* enzyme and separated from each other.[267] Both were required for the hydrolytic desulphation of the products of 'chondroitinase ABC' action, but did not degrade polymeric chondroitin sulphates nor the tri- to hexa-saccharides obtained from hyaluronidase digests of chondroitin 4- and 6-sulphates or 2-acetamido-2-deoxy-D-galactose 4- or 6-sulphates. Chondro-4-sulphatase and chondro-6-sulphatase catalysed the desulphation of the saturated and 4,5-unsaturated disaccharide 4-sulphates and corresponding 6-sulphates, respectively. The latter enzyme catalysed the hydrolysis of the 6-sulphate of 2-acetamido-2-deoxy-D-galactose 4,6-di-sulphate.

This knowledge of the properties of 'chondroitinase ABC', 'chondroitinase AC', chondro-4-sulphatase, and chondro-6-sulphatase has been exploited in the development of new micromethods for measurement of as little at 3 μg. of chondroitin 4-sulphate, chondroitin 6-sulphate, and dermatan sulphate in mixtures.[268]

Another, unsaturated disaccharide, identified as 2-acetamido-2-deoxy-3-(β-D-*glycero*-hex-4-eneosyl uronic acid), 4,6-di-*O*-sulpho-D-galactose, has been isolated from squid cartilage on incubation with 'chondroitinase

[265b] E. H. Makarem and R. S. Berk, *J. Infectious Diseases*, 1968, **118**, 427.
[266] P. F. Lloyd and R. J. Fielder, *Biochem. J.*, 1968, **109**, 14P.
[267] T. Yamagata, H. Saito, O. Habucki, and S. Suzuki, *J. Biol. Chem.*, 1968, **243**, 1523.
[268] H. Saito, T. Yamagata, and S. Suzuki, *J. Biol. Chem.*, 1968, **243**, 1536.

ABC'.[269] Similar treatment of dermatan sulphate from bovine lung and pig skin gave the isomeric disulphated disaccharides, 2-acetamido-2-deoxy-3-*O*-(2- or 3-*O*-sulpho-β-D-*glycero* hex-4-eneosyl uronic acid)-6-*O*-sulpho-D-galactose, which had previously been reported in enzymic digests of shark chondroitin sulphate. These three unsaturated disaccharides were separated by paper chromatography thus offering a method for detecting slight variations in the type of sulphate linkage in different preparations of chondroitin sulphate.

Information relating to the distribution of sulphate groups in chondroitin sulphates has been obtained by electrophoresis after hyaluronidase digestion.[270] Oversulphation, that is more than one sulphate residue per disaccharide 'repeating unit', has been found as a general characteristic of the class *Chondricthyes* and oversulphated oligosaccharides have also been found in squid and coelacanth cartilage, acellular bone of cod, and shark cornea.

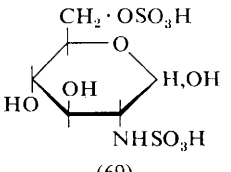
Heparin.—A heparin–protein complex has been isolated from ox-liver by extraction with 1% cetylpyridinium chloride (CPC) in 2M-potassium chloride, dilution of the supernatant to 0·9M-potassium chloride, removal of the CPC by treatment with chloroform–pentan-1-ol, and precipitation of the heparin with alcohol.[271] Interaction of the polysaccharide with insoluble collagen was thus prevented by blocking the sulphate groups by CPC. A new method for the isolation of heparin from tissues has been described based on precipitation with potassium acetate.[272] Fractionation of mixtures of acidic glycosaminoglycans was determined mainly by the ratio of sulphate ester to carboxyl content, and only to a small extent on molecular weight; the lower the ratio of sulphate to carboxyl in a fraction, the higher the concentration of potassium acetate required to precipitate it. Heparin was selectively precipitated at room temperatures by $>1{\cdot}5$M-potassium acetate.

G.l.c. analysis of formic acid hydrolysates of heparin demonstrated the presence of glucuronic acid, iduronic acid, and xylose.[273] Other workers characterised L-iduronic acid, as its crystalline brucinium salt, from acid hydrolysates of highly purified heparin, and showed that it was not an artifact of hydrolysis.[274] N.m.r. studies of commercial heparin showed [274a] at least two types of heparin that were differentiated by small but distinct variations in the proton signals and by the presence of acetyl residues in

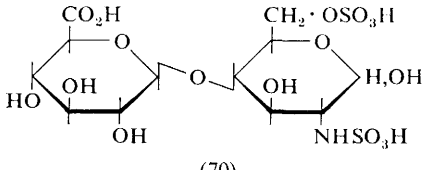
[269] S. Suzuki, H. Saito, T. Yamagata, K. Anno, N. Seno, Y. Kawai, and T. Furuhashi, *J. Biol. Chem.*, 1968, **243**, 1543.
[270] M. B. Mathews and L. Decker, *Biochim. Biophys. Acta*, 1968, **156**, 419.
[271] A. Serafini-Fracassini and J. J. Durward, *Biochem. J.*, 1968, **109**, 693.
[272] J. E. Scott, T. E. Stacey, and M. J. Tigwell, *Biochem. J.*, 1968, **108**, 50P.
[273] B. Radhakrishnamurthy, E. R. Dalferes jun., and G. S. Berenson, *Analyt. Biochem.*, 1968, **24**, 397.
[274] M. L. Wolfrom, S. Honda, and P. Y. Wang, *Chem. Comm.*, 1968, 505.
[274a] A. S. Perlin, M. Mazurek, L. B. Jaques, and L. W. Kavanagh, *Carbohydrate Res.*, 1968, **7**, 369.

one of the types. The authors also concluded that at least three kinds of sugar residues were present in all the heparin samples.

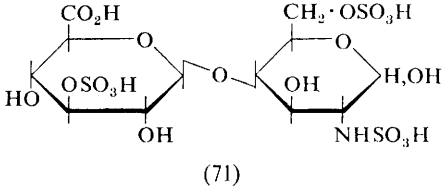
Linkage analysis of heparin and related acidic glycosaminoglycans has been facilitated by relatively mild hydrolysis of the *N*-(2,4-dinitrophenyl)-derivatives.[275] Thus, hydrolysis (N-sulphuric acid, 90 min., 100°) of this derivative of *N*-desulphated heparin liberated, among other products, the *N*-(2,4-dinitrophenyl) derivatives of 2-amino-2-deoxy-D-glucose (1%), and 4-*O*-α-(2-amino-2-deoxy-D-glucosyl)-D-glucopyranuronic acid (8%) together with a monosulphated tetrasaccharide (26%). Several sulphated di- and oligo-saccharides were produced among other products, by degradation of heparin with enzymes from an adapted *Flavobacterium heparinum*.[275a] Structures (69–71) were proposed for some of the products and the tri-substituted disaccharide (71) was considered to be a fundamental unit in the heparin molecule.



(69)



(70)



(71)

N-Acetylated, rather than *N*-sulphated, 2-amino-2-deoxy-D-glucose residues have been shown to be components of the heparin–protein linkage region.[276] Treatment of heparin with nitrous acid followed by acid hydrolysis liberated oligosaccharides (72–75) together with an oligosaccharide

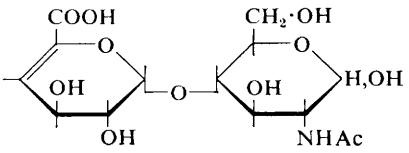
GA→Gal	(72)
GA→GN→$(GA)_2$→Gal	(73)
GA→GN	(74)
GA→GN→ GA →Gal	(75)
GA→GNAc	(75a)
GA→$GNSO_3H$	(76)

[275] P. F. Lloyd and B. Evans, *Carbohydrate Res.*, 1968, **8**, 372.
[275a] C. P. Dietrich, *Biochem. J.*, 1968, **108**, 647.
[276] U. Lindahl, *Biochim. Biophys. Acta*, 1968, **156**, 203.

which contained galactose, xylose, glucuronic acid, and iduronic acid. Oligosaccharide (73) may represent a branch point in the polysaccharide.

Heparitin Sulphate.—Characterisation of the products obtained on treatment of heparitin sulphate with nitrous acid indicated that the polysaccharide contained multiple, alternating sections containing 2-acetamido-2-deoxy-*O*-(glucosyluronic acid)-D-glucose (75a) or 2-deoxy-*O*-(glucosyluronic acid)-2-sulphamino-D-glucose (76) with each separate section containing either only *N*-acetyl or *N*-sulphate residues.[277] Some variation in the size of the sections was indicated. Six to seven units of (75a) were located at the protein end of the chain, and single units of (76) were distributed either within the interior of the polysaccharide chain or at the non-reducing terminus. The distribution of *N*-sulphated regions (76) was 'random' except near the carbohydrate–peptide linkage region.

The isolation in 10% yield, of the non-sulphated disaccharide (77) and the polymer homologous tetrasaccharide, from heparitin sulphate after treatment with a heparin eliminase from *Flavobacteria*, confirmed that at least



(77)

two or more *N*-acetylated units occurred in sequence in the polysaccharide and that the linkage in the non-sulphated portion of heparitin sulphate was mainly α-(1 → 4).[278]

Dermatan Sulphate.—Glycopeptides isolated from the carbohydrate–protein linkage region of pig skin dermatan sulphate following treatment with testicular hyaluronidase were identified[279] as (GA—GNAc)$_n$—GA—Gal—Gal—Xyl—Ser-(peptide) where $n = 1$ or 2. Other workers[280] have shown that the carbohydrate–peptide linkage region in dermatan sulphate includes the sequence xylosyl—serinyl—glycine.

A unique distribution of D-glucuronic acid residues has been found[281] in dermatan sulphate from hog intestinal mucosa by comparison with that found in the same polysaccharide from pig skin and umbilical cord. Thus, the hyaluronidase-susceptible bonds in the polysaccharide from the mucosa were located exclusively in the non-reducing, terminal portion of the

[277] J. A. Cifonelli, *Carbohydrate Res.*, 1968, **8**, 233.
[278] A. Linker and R. Hovingh, *Biochim. Biophys. Acta*, 1968, **165**, 89.
[279] L.-Å. Fransson, *Biochim. Biophys. Acta*, 1968, **156**, 311.
[280] A. Bella jun., and I. Danishefsky, *J. Biol. Chem.*, 1968, **243**, 2660.
[281] L.-Å. Fransson, *Arkiv Kemi.*, 1968, **29**, 95.

molecule, whereas such linkages were randomly distributed in the polysaccharide from the latter two sources. Certain structural differences were found, however, in the dermatan sulphates from pig skin and umbilical cord.[282] The polysaccharide from the latter source contained both L-iduronic acid and D-glucuronic acid units and a considerable proportion of 2-amino-2-deoxy-D-galactose 6-sulphate, whereas in the polysaccharide from pig skin the 2-amino-2-deoxy-D-galactose residues were exclusively 4-sulphated. The 6-sulphated residues were preferentially located adjacent to D-glucuronic acid residues and the 4-sulphated residues were prevalent in L-iduronic acid-containing sections of the molecule. Some of the 2-amino-2-deoxy-D-galactose residues adjacent to L-iduronic acid residues, however, were 6-sulphated. Hybrid octasaccharides obtained on treatment of umbilical cord dermatan sulphate with hyaluronidase had the monosaccharide sequence:

$$\underset{\text{6-Sulphate}}{\text{GA}\rightarrow\text{GalNAc}}\rightarrow\underset{\text{4/6-Sulphate}}{\text{IdA}}\rightarrow\underset{\text{4/6-Sulphate}}{\text{GalNAc}}\rightarrow\underset{\text{6-Sulphate}}{\text{IdA}}\rightarrow\text{GalNAc}\rightarrow\text{GA}\rightarrow\text{GalNAc}$$

One-third of the two-L-iduronic acid-containing internal disaccharides were also sulphated at C-6. D-Glucuronic acid residues were shown to be rare in the vicinity of the carbohydrate–peptide linkage region of umbilical cord dermatan sulphate.

Keratan Sulphate.—Methylation studies of keratan sulphate from old human rib cartilage, and of its desulphated analogue, showed [283] that the main repeating unit was 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-acetyllactosamine) polymerised *via* a (1 → 3)-linkage to galactose as had been found in bovine corneal keratan sulphate. The polysaccharide isolated from cartilage, however, was more highly branched than that from cornea. 2-Acetamido-2-deoxy-D-glucose and/or D-galactose residues were substituted at C-6 by sulphate ester groups and the polymer also contained sialic acid, L-fucose, and D-mannose.

Hyaluronic Acid.—The enzymic degradation of hyaluronic acid, but not alginic acid nor pectin, was demonstrated on incubation with human serum at pH 4·5.[284] Incubation of hyaluronic acid with rat liver lysosomes caused 85% degradation of hyaluronic acid to an equimolar mixture of D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose with 15% of the molecule remaining as hyalobiouronic acid.[184] Other workers [284a] demonstrated that purified lysosomes degraded a protein–polysaccharide from cartilage and that the major action of leucocyte lysosomes was trypsin-like, whereas that of liver lysosomes was hyaluronidase-like.

[282] L.-Å. Fransson, *J. Biol. Chem.*, 1968, **243**, 1504.
[283] V. P. Bhavanandan and K. Meyer, *J. Biol. Chem.*, 1968, **243**, 1052.
[284] A. Herp, J. de Filippi, and J. Fabianek, *Biochim. Biophys. Acta*, 1968, **158**, 150.
[284a] G. Weissmann and I. Spilberg, *Arthritis Rheum.*, 1968, **11**, 162.

Biosynthesis of Mammalian Glycosaminoglycans.—An enzyme isolated from mast cell tumours catalysed the reaction

D-Fru 6-phosphate + L-glutamine ⇌ D-GN 6-phosphate + L-glutamate

which is one of the steps in the biosynthesis of heparin.[285] The *in vitro* biosynthesis of chondroitin sulphate by human leukocytes [286] and leukemic cells [287] has been demonstrated. Sulphotransferase and glycosaminoglycan-polymerising activities, as well as chondroitin sulphate, were found in a microsomal preparation from chick embryo cartilage.[288] Hyaluronidase, chondroitin sulphate sulphohydrolase, *N*-acetyl-β-D-glucosaminidase, β-D-glucuronidase, cathepsin D, and an acid carboxypeptidase were purified from bovine arterial tissue, and their involvement in the degradation of chondroitin sulphate in arterial tissue has been discussed.[289, 290] Some degree of interaction between the biosynthesis of collagen and chondroitin sulphate has been noted.[291] *In vitro* studies showed that rats were capable of concentrating *ca.* 1% of isotopically labelled chondroitin 4- and 6-sulphates and dermatan sulphate in their liver lysosomes, but that chondroitin 4-sulphate was depleted from the lysosomes four days after injection, whereas, after the same time period, one-third of the dermatan sulphate remained in the cellular particles.[292] The potential pathological importance of these findings, *e.g.* in Hurler's syndrome, was emphasised. The effect of vitamin A on the acidic glycosaminoglycans of lung tissue has been investigated.[293]

Results of studies on the biosynthesis of chondroitin sulphate–protein complexes in calf costal cartilage were consistent with a mechanism for chondroitin sulphate synthesis in which elongation of the polysaccharide chain was coupled to its sulphation.[294, 295] Evidence was also obtained [295] for the presence of a control system that related synthesis of the sulphated polysaccharide to the formation of the polysaccharide–protein linkage region. Studies of the formation of chondroitin sulphate by a microsomal preparation of chick embryo cartilage suggested [295a] that polymerisation and sulphation took place in close proximity in the cell.

No mechanism similar to that which occurs in the synthesis of bacterial cell walls was demonstrated in studies of the synthesis of hyaluronic acid by

[285] I. Danishefsky and L. Deutsch, *Biochim. Biophys. Acta*, 1968, **151**, 529.
[286] I. Olsson, S. Gardell, and S. Thunell, *Biochim. Biophys. Acta*, 1968, **165**, 309.
[287] I. Olsson, *Biochim. Biophys. Acta*, 1968, **165**, 324.
[288] S. DeLuca and J. E. Silbert, *J. Biol. Chem.*, 1968, **243**, 2725.
[289] E. Held, O. Hoefele, G. Reich, U. Stein, E. Werries, and E. Buddecke, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 244.
[290] H. Kresse and E. Buddecke, *Z. Klin. Chem. Klin. Biochem.*, 1968, **6**, 251.
[291] B. Robosová-Čmuchalová and J. P. Bentley, *Biochem. Pharmacol., Suppl.*, 1968, 315.
[292] N. N. Aronson jun., and E. A. Davidson, *J. Biol. Chem.*, 1968, **243**, 4494.
[293] L. DeLuca and G. Wolf, *Arch. Biochem. Biophys.*, 1968, **123**, 1.
[294] T. O. Kleine and H. Hilze, *Z. Physiol. Chem.*, 1968, **349**, 1027.
[295] T. O. Kleine and H. Hilze, *Z. Physiol. Chem.*, 1968, **349**, 1037.
[295a] J. E. Silbert and S. DeLuca, *Biochem. Biophys. Res. Comm.*, 1968, **31**, 990.

Group A *Streptococci*.[295b] Thus, no evidence was obtained for lipid intermediates which might be involved in the utilisation of two intracellular nucleotides for the synthesis of an extracellular polysaccharide.

Physicochemical Studies.—The interaction between heparin DNA and various cations in aqueous solution has been studied by pulse radiolysis.[296–298] The strengths of the metachromatic reactions of cationic dyes in approximately 1 : 1 complexes with various acidic polysaccharides have been measured.[298a]

The molecular weight of shark chondroitin 6-sulphate in 0·6M-sodium chloride and 0·2M-calcium chloride was calculated (sedimentation equilibrium) as $5{\cdot}2 \times 10^4$ and $5{\cdot}5 \times 10^4$, respectively.[299] O.r.d. and c.d. studies of chondroitin 6-sulphate showed a strong, negative, optically active band near 210 mμ which was attributed to the presence of carboxylate and *N*-acetyl groups. The authors suggested that the *N*-acetyl group contributed more to the rotations of chondroitin 6-sulphate than did carboxylate, but that acidification of the carboxylate group largely accounted for the change in magnitude and position of the c.d. bands that was observed at low pH.

Studies of the molecular weight heterogeneity of heparin by sedimentation equilibrium showed an average *Z* average : weight average ratio of *ca.* 1·8.[300] The molecular weight of fractionated bovine heparin, calculated from low angle *X*-ray scattering data, was shown to be 12,900 in excellent agreement with values (12,600 and 12,500) obtained for the same fraction from sedimentation equilibrium and viscosity studies, respectively.[300a] The calculated persistence length and radius of gyration of the molecule were 21·1 Å and 35·9 Å, respectively, and the molecule in water at room temperature was best described as a Gaussian coil molecule.

Destruction of approximately half the anti-coagulant activity of heparin by acid hydrolysis caused little change in molecular weight thus indicating that the initial stage of hydrolysis might involve the formation of internal esters.[300] Similar destruction of coagulant activity by oxidation with periodate, however, was accompanied by a reduction in molecular weight with loss of nitrogen and oxidative cleavage.

Two major types of protein–polysaccharide complex isolated from the nucleus pulposus of whale invertebral discs behaved differently on treatment

[295b] N. Ishimoto and J. L. Strominger, *Biochim. Biophys. Acta*, 1967, **148**, 296.
[296] E. A. Balazs, J. V. Davies, G. O. Phillips, and D. S. Schuefele, *Biochem. Biophys. Res. Comm.*, 1968, **30**, 386.
[297] E. A. Balazs, J. V. Davies, G. O. Phillips, and D. S. Schuefele, *J. Chem. Soc.* (*C*), 1968, 1424.
[298] E. A. Balazs, J. V. Davies, G. O. Phillips, and D. S. Schuefele, *J. Chem. Soc.* (*C*), 1968, 1429.
[298a] A. L. Stone, *Biochim. Biophys. Acta*, 1967, **148**, 193.
[299] E. J. Eyring and J. T. Yang, *Biopolymers*, 1968, **6**, 691.
[300] E. Brasswell, *Biochim. Biophys. Acta*, 1968, **158**, 103.
[300a] S. S. Stivala, M. Herbst, O. Kratky, and I. Pilz, *Arch. Biochem. Biophys.*, 1968, **127**, 795.

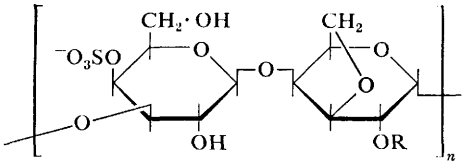
with disulphide reducing agents.[301] $\bar{M}_w$ of the fraction that contained cystine residues changed from $8{\cdot}5 \times 10^6$ to $6{\cdot}6 \times 10^5$ on treatment with dithiothreitol, whereas the fraction that lacked cystine was unchanged on such treatment. The estimated molecular weight of the protein–polysaccharide light fraction from bovine nasal septum was *ca.* $1{\cdot}8 \times 10^6$ after disaggregation. The fraction contained no disulphide or salt linkages.[302]

Physico-chemical studies of ox synovial fluid hyaluronic acid suggested that protein might effect the interaction of individual molecules in solution by forming loose, intermolecular links between them.[303] A method was described for measuring, approximately, the thermodynamic non-ideality of a solute from the shape of its Schlieren curve at sedimentation equilibrium in a density gradient. The formation of complexes between hyaluronic acid and various steroids has been demonstrated.[304] Complex formation with cortisone increased the rate of buffer flow through the hyaluronate solution, changed its rheological properties from dilatant to thixotropic, and increased its viscosity. The formation of soluble complexes of hyaluronic acid and bovine serum albumin at pH 5·3, 7·1, 8·5, and 8·6 has also been demonstrated.[305]

Polysaccharide Sulphates and Other Polysaccharides from Seaweeds

Carrageenans.—Further studies by the Edinburgh group have led to the concept of a masked repeating structure in carrageenans. Thus κ-carrageenan was shown to contain an alternating arrangement of 3-linked β-D-galactopyranosyl residues and 4-linked α-D-galactopyranosyl residues in which the 3-linked units were present only as the 4-sulphate, and the 4-linked units included the 3,6-anhydride 2-sulphate, the 6-sulphate, and the 2,6-disulphate.[306] On treatment with alkali and borohydride κ-carrageenan was converted into a molecule which contained equimolar proportions of D-galactose and 3,6-anhydro-D-galactose units and which, on methanolysis, gave 3,6-anhydro-4-(*O*-β-D-galactopyranosyl)-D-galactose dimethyl acetal (carrabiose dimethyl acetal) in a yield which was close to that calculated for a perfectly alternating polymer. Methylation studies of this polymer (78) showed that virtually every D-galactose unit was 4-*O*-sulphated and that approximately every seventh 3,6-anhydro-D-galactose unit was 2-*O*-sulphated. This masked repeating type of structure occurred generally in κ-carrageenans from *Chondrus crispus* and *Gigartina* species with most, and possibly all, of the variations between samples in the 4-linked units.[307]

[301] H. Hashimoto and J. L. Ludowieg, *Biochemistry*, 1968, **7**, 2469.
[302] E. J. Eyring and J. T. Yang, *J. Biol. Chem.*, 1968, **243**, 1306.
[303] P. Silpananta, J. R. Dunstone, and A. G. Ogston, *Biochem. J.*, 1968, **109**, 43.
[304] N. Keller, *Biochim. Biophys. Acta*, 1967, **148**, 757.
[305] W. Niedermeier and E. S. Gramling, *Carbohydrate Res.*, 1968, **8**, 317.
[306] N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J. Chem. Soc.* (*C*), 1968, 596.
[307] N. S. Anderson, T. C. S. Dolan, A. Penman, D. A. Rees, G. P. Mueller, D. J. Stancroff, and N. F. Stanley, *J. Chem. Soc.* (*C*), 1968, 602.

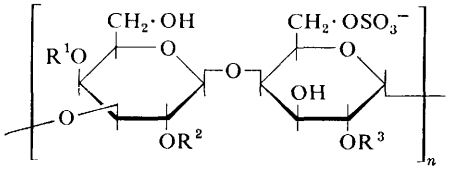


R = SO_3^- in approximately every seventh anhydro-sugar residue

(78)

A sample from *Chondrus* was separated into subfractions which differed in the relative proportions of the various 4-linked units, and an i.r. absorption band at *ca.* 805 cm^{-1} was shown to be characteristic of 3,6-anyhdro-D-galactose sulphate. Gel formation was enhanced by removal of the 6-sulphate, with concomitant conversion to a 3,6-anhydro-D-galactose residue, but was much less sensitive to an excess of 2-sulphate.

Two potassium chloride-soluble carrageenans, designated λ and μ, were also shown to have masked repeating structures based on alternately 3-linked and 4-linked galactose residues, but had important differences in their sulphation patterns and 3,6-anhydride content [308] [see (79)]. Treatment



μ-Carrageenan; $R^1 = SO_3^-$; $R^2 = R^3 = H$

λ-Carrageenan; $R^1 = H$; $R^2 = SO_3^-$(70%) or H (30%); $R^3 = SO_3^-$

(79)

of a mixture of λ- and μ-carrageenans with alkali and borohydride gave potassium chloride-soluble and -insoluble products from the λ- and μ-polysaccharides, respectively. Lawson and Rees [309] obtained results consistent with a repeating structure of alternately α-(1 → 3)- and β-(1 → 4)-linked D-galactopyranose residues for λ-carrageenan from re-investigating the products of acetolysis and subsequent deacetylation. A trisaccharide thus obtained, and which was previously assigned the structure *O*-α-D-galactopyranosyl-(1 → 3)-*O*-α-D-galactopyranosyl-(1 → 3)-D-galactose (K. Morgan and A. N. O'Neill, *Canad. J. Chem.*, 1959, **37**, 1201), was shown to be *O*-α-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-galactose.

[308] N. S. Anderson, T. C. S. Dolan, C. J. Lawson, A. Penman, and D. A. Rees, *Carbohydrate Res.*, 1968, 7, 468.

[309] C. J. Lawson and D. A. Rees, *J. Chem. Soc.* (*C*), 1968, 1301.

Similar effects on the physical properties of some carrageenans following treatment with alkali were reported by Haug and co-workers.[310] The main effect of alkali was on the fraction originally precipitated at intermediate potassium chloride concentrations and led to a higher content of 3,6-anhydro-D-galactose residues, higher gel strength, and lower solubility in potassium chloride solutions. Chemical differences other than the 3,6-anhydro-D-galactose content, however, also controlled the solubility level.

Alginic Acid.—Painter and co-workers,[311] in a fundamental computer study of the changes in composition-distribution occurring during random depolymerisation of a binary linear heteropolysaccharide, concluded that the 'measure of agreement between the actual behaviour of alginate when subjected to limited depolymerisation by acid and that predicted theoretically on the assumption of random cleavage, is sufficiently good ... to encourage the belief that a first step has been taken towards a description of alginate structure in terms of the substrate specificities of the alginate polymerase system in the living alga'. A detailed study was made of the dependence upon uronic acid composition of some ion-exchange properties of alginates.[312] The decrease in viscosity and colour reaction with 2-thiobarbituric acid that occur on degradation of alginate at pH 11 was attributed to a β-alkoxy-elimination reaction.[313] From the results of light scattering studies Smidsrød and Haug[314] concluded that the alginate molecules in solution in 0·09M-sodium chloride and 0·01M-sodium fluoride were best described as very extended random coils with partial free drainage. The relationships between intrinsic viscosity, molecular weight, and radius of gyration were also described.

Other Polysaccharides from Seaweeds.—Water-soluble polysaccharides of the red alga *Laurencia pinnatifida* were identified as a glucan and a sulphated galactan.[315] The latter polysaccharide contained 1,3-linked D-galactose and 6-*O*-methyl-D-galactose units, 1,4-linked L-galactose and 2-*O*-methyl-L-galactose units together with units of 3,6-anhydro-L-galactose, 3,6-anhydro-2-*O*-methyl-L-galactose, and sulphate.[316] 1,3-Linked D-glucuronic acid residues and xylose in the polysaccharide preparation might have been constituents of an associated polysaccharide. A sulphated galactan which liberated D-galactose and D-xylose (3 : 1) on complete hydrolysis was also isolated from the red alga *Laingia pacifica*.[317] The polysaccharide contained *ca.* 10% of 3,6-anhydro-sugar and the majority of the 12% of sulphate ester residues were stable to alkali. A second polysaccharide isolated from the

[310] O. Smidsrød, B. Larson, A. J. Pernas, and A. Haug, *Acta Chem. Scand.*, 1967, **21**, 2585.
[311] T. Painter, O. Smidsrød, B. Larsen, and A. Haug, *Acta Chem. Scand.*, 1968, **22**, 1637.
[312] O. Smidsrød, and A. Haug, *Acta Chem. Scand.*, 1968, **22**, 1989.
[313] A. Haug, B. Larsen, and O. Smidsrød, *Acta Chem. Scand.*, 1967, **21**, 2859.
[314] O. Smidsrød and A. Haug, *Acta Chem. Scand.*, 1968, **22**, 797.
[315] D. M. Bowker and J. R. Turvey, *J. Chem. Soc.* (*C*), 1968, 983.
[316] D. M. Bowker and J. R. Turvey, *J. Chem. Soc.* (*C*), 1968, 989.
[317] N. K. Kochetkov, A. I. Usov, and L. Miroshnikova, *Zhur. obshchei Khim.*, 1967, **37**, 792.

same alga was characterised as an α-glucan with an amylopectin type structure.

Partial hydrolysis with acid of the sulphated glucuronoxylofucan component of the cell-wall of *Ascophyllum nodosum* liberated 3-*O*-(β-D-glucopyranosyluronic acid)-L-fucose as the major component together with 3-*O*-β-D-xylopyranosyl-L-fucose and 4-*O*-α-L-fucopyranosyl-D-xylose.[318] The sulphate ester groups were resistant to alkali. The polysaccharide had a branched structure with 1,4-linked xylose residues near the periphery, at least some of the D-glucuronic acid residues 1,4-linked, and some of the fucose residues susceptible to periodate oxidation.

A highly sulphated ($Na^+SO_3^-$, 27·5%), methylated galactan, designated aeodan, isolated from *Aeodes orbitosa*, was shown to contain D-galactose (58%), 2-*O*-methyl-D-galactose (10%), and glycerol.[319] Methylation and periodate oxidation studies of the native and desulphated polysaccharides indicated the presence of (1 → 3)- and (1 → 4)-glycosidic linkages and sulphate residues at C-2 and C-6. Treatment with alkali and borohydride showed the presence of *ca.* 5·7% of (1 → 2)- or (1 → 4)-linked galactose 3- or 6-sulphate residues. 2,6- and 4,6-Di-*O*-methyl-D-galactose, 2,3,6- and 2,4,6-tri-*O*-methyl-D-galactose, and 2,3,4,6-tetra-*O*-methyl galactose were obtained on hydrolysis of methylated, desulphated galactan. 4-*O*-Methyl-L-galactose, 6-*O*-methyl-D-galactose, and the disaccharides 4-*O*-β-D-galactopyranosyl-D-galactose and 3-*O*-D-galactopyranosyl-D-galactose were isolated from the galactan by hydrolysis with acid.[319a]

An extracellular agarase from *Cytophaga* species (N.C.M.B. 1327), identified as an endoenzyme, degraded agarose to give oligosaccharides as the major products with only small amounts of disaccharide (neoagarobiose) and no monosaccharides.[320] By contrast, degradation of agarose by the intracellular enzyme from the same source produced some monosaccharides.

[318] E. Percival, *Carbohydrate Res.*, 1968, **7**, 272.
[319] J. R. Nunn and H. Parolis, *Carbohydrate Res.*, 1968, **6**, 1.
[319a] J. R. Nunn and H. Parolis, *Carbohydrate Res.*, 1968, **8**, 361.
[320] M. Duckworth and J. R. Turvey, *Biochem. J.*, 1968, **109**, 6P.

7

Chemical Synthesis and Modification of Polysaccharides

6-*O*-Methylacryloyl-D-galactose was polymerised readily in water to give a water-soluble polymer, and in the presence of ammonium persulphate to give a water-insoluble, cross-linked hydrogel.[321]

Methods were described [322] for the synthesis of methylated Sephadex and various lipophilic Sephadex ion-exchangers. Oxidation, with DMSO and acetic anhydride, of a dextran 2,4-phenylboronate produced a 'ketodextran' (0·29 C=O per hexose unit) with most of the carbonyl groups at C-3.[323] Another 'ketodextran', with a similar distribution of carbonyl groups, was obtained on oxidation of an unprotected dextran with the same reagents. Reduction, with sodium borohydride, of either of the 'ketodextrans' gave modified dextrans in which *ca.* 25% of the original D-glucose residues had been converted to D-allose residues and *ca.* 2·5% and 3%, respectively, to D-mannose and D-galactose. Oxidation of 6-*O*-trityl amylose with DMSO and acetic anhydride at 25° for 20 hr. was shown [324] to give a product in which oxidation had occurred predominantly at C-2. Thus, reduction of the oxidised derivative with sodium borohydride, followed by sequential detritylation and acid hydrolysis, gave D-glucose and D-mannose and a small amount of a third component. Oximation of the oxidised polysaccharide, followed by sequential reduction with lithium aluminium hydride, detritylation, and acid hydrolysis, gave 2-amino-2-deoxy-D-glucose and D-glucose.

The preparation of carboxymethylchitin with one carboxymethyl group per 'repeating unit' has been reported.[325]

Studies of the kinetics of xanthation of cellulose in a homogeneous medium showed [325a] that xanthate formation was a second-order reaction. The ratio of substituents in the 2-*O*-, 3-*O*-, and 6-*O*-positions, resulting from the base-catalysed reaction of sodium allyl sulphate with cotton cellulose to a degree of substitution of 0·07, was 0·7 : 0·2 : 1·0.[326]

[321] W. A. P. Black, J. A. Colquhoun, and E. T. Dewar, *Makromol. Chem.*, 1968, **117**, 210.
[322] E. Nyström, *Arkiv Kemi*, 1968, **29**, 99.
[323] A. N. de Belder, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1968, **22**, 949.
[324] M. L. Wolfrom and P. T. Wang, *Chem. Comm.*, 1968, 113.
[325] R. Trujillo, *Carbohydrate Res.*, 1968, **7**, 483.
[325a] L. Anderson, O. Samuelson, and B. Törnell, *Makromol. Chem.*, 1968, **119**, 133,
[326] D. E. Hoiness, C. P. Wade, and S. P. Rowland, *Canad. J. Chem.*, 1968, **46**, 667.

Methylation studies of pustulan [β-D-(1 → 6)glucan] and of various glucosides and 1,6-anhydro-D-glucopyranose, using dimethyl sulphate in 19% aqueous sodium hydroxide, have been investigated in order to evaluate the relative importance of factors such as H-bonding, electronic and steric effects, in determining the differences in reactivity between hydroxy-groups at C-2, C-3, and C-4 of an anhydroglucose unit in dextran.[326a] The author concluded that no special effects were needed to account for the low reactivity of the C-3-hydroxy-group, but that the reactivity of the C-4-hydroxy-group did seem to be dependent upon the nature of the substituent at C-6. It was tentatively suggested that this effect might be attributed to H-bonding.

In further studies of radiation-induced reactions with cellulose it was shown[327] that styrene and methyl methacrylate readily grafted on to cellulose in acetone, dioxan, and DMF solvents. The mechanism of the co-polymerisation with styrene in non-wetting solvents has been studied.[327a]

Acid- and heat-catalysed dextrinisation of amylopectin in the presence of D-galactose produced a heterogeneous co-dextrin (average degree of polymerisation = 37) with 13·5% D-galactose incorporated into a polymer.[328] Partial hydrolysis of the product liberated, among other products, 6-*O*-α-D-glucopyranosyl-D-galactose. The authors concluded that the reaction took place with a drastic reduction in molecular weight and the development of a highly branched structure. Studies of the action of chlorine on wheat flour polysaccharides showed[328a] that, under dry conditions, chlorinolysis proceeded with depolymerisation through glycosidic cleavage *via* an intermediate chloroxonium ion. Under neutral to strongly acidic conditions only, a minor reaction was the oxidation of hydroxy-groups at C-2, C-3, and C-6.

trans-Fused cyclic carbonates of certain carbohydrates readily substituted on to starch under basic conditions.[329] 'Dextran carbonates' were shown to react to give polysaccharide copolymers.

Malto-oligosaccharides (degree of polymerisation 1–16), in which the hydroxy-group at C-4 only of the terminal, non-reducing residues was methylated, have been prepared from amylose, and used in further studies of the mechanism of the action of β-amylase.[330]

[326a] B. Norrman, *Acta Chem. Scand.*, 1968, **22**, 1623.
[327] S. Dilli and J. L. Garnett, *Austral. J. Chem.*, 1968, **21**, 1827.
[327a] S. Dilli and J. L. Garnett, *Austral. J. Chem.*, 1968, **21**, 397.
[328] M. H. Fischer, Y. Ghali, and F. Smith, *Cereal Chem.*, 1968, **45**, 421.
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[329] W. M. Doane, B. S. Shasha, E. I. Stout, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1968, **8**, 266.
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8
Physicochemical Studies

Some general rules governing hydrogen bonding at the ring oxygen atoms of furanosides, pyranosides, and the bridge oxygen atoms of glycosides have been formulated from existing data on crystal structures of carbohydrates.[331] The empirical rule relating the orientation (axial or equatorial) and chemical shift of *O*-acetate methyl and *O*-methyl signals in the n.m.r. spectra of monosaccharide derivatives has been shown to apply to the conformational analysis of monosaccharide moieties in homo- and heteropolysaccharides.[332] These results showed that the repeating uronic acid moiety in pectic acid exists in the 1*C*-conformation, and allowed the preferred conformation of the hexopyranosyluronic acid moieties in chondroitin 6-sulphate and heparin to be depicted.[332]

The number-average degree of polymerisation of linear maltodextrins and amylose has been determined by analysis of the products of amylolytic digestion.[333] Measurement of D-glucose, by reaction with D-glucose oxidase, in the products of β-amylolysis enabled calculation of $\bar{M}_n$, when compared with the total D-glucose content obtained by reaction with amyloglucosidase. The distribution of molecular weight in amylose samples obtained by the leaching and dispersion of potato starch has been studied.[334] An exponential distribution of molecular weights expected of a random AA polymer was found in leached material, whereas the total material showed a broader distribution compatible with the presence of a branched fraction.

Examination of the hydrodynamic properties of a subfractionated linear amylose has enabled a study of the conformation of amylose in neutral aqueous salt solution to be made.[335] It was concluded that the view of amylose as a molecule having a number of rigid helical segments has no experimental basis. The hydrodynamic behaviour of native amylose in thermodynamically good solvents indicated that the amylose molecules were extended as a result of solute–solvent interactions.[336] The same basic skeletal structure was apparent in each solvent, a random coil with little

[331] M. Sundaralingam, *Biopolymers*, 1968, **6**, 189.
[332] S. Hirano, M. Manabe, N. Miyazaki, and K. Onedera, *Biochim. Biophys. Acta*, 1968, **156**, 213.
[333] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1968, **6**, 177.
[334] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1968, **6**, 171.
[335] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1968, **7**, 349.
[336] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1968, **7**, 414.

helical character being consistent with the experimental data. The transition from helix to coil at pH 12 for amylose, amylopectin, and glycogen has been examined [337] with the conclusion that a helix existed in amylopectin and glycogen but not in α-(1 → 6)-linked dextran. The stabilising force for the helix was postulated as hydrogen bonding between C-2- and C-3-hydroxy-groups of adjacent glucopyranose units. The stability of the helix of amylose and amylopectin in DMSO and water solutions indicated that DMSO or DMSO,$2H_2O$ stabilised the starch helix.[338] The affect of aqueous salt solutions on the conformation and thermal motion of molecules such as starch has been studied.[338]

The anomalous characteristics of amylopectin fractions from starches of high amylose content were explained by the presence of contaminating, short-chain, linear material.[339] Failure of the linear material to complex with butanol, owing to a low degree of polymerisation, accounted for the contamination. Sedimentation studies on amylose acetate in nitromethane solution suggested that molecules were extended as a result of solute–solvent interactions rather than a high degree of steric hindrance.[340]

O.r.d. and c.d. measurements have been made on iodine–polysaccharide complexes.[341]

The degree of polymerisation of amylose on a microscale has been determined approximately by measurement of the concentration of free iodine in a solution of the amylose–iodine complex.[341a]

Both ions in orientated fibres of potassium bromide–amylose were located in a water-like environment. The oxygens O(2), O(3), and O(4) from glucose residues on adjacent chains co-ordinated around the potassium cation.[342] No evidence for methanol, ethanol, or n-propanol structures similar to those shown by n-butanol was found in the analysis of the structure of amylose–n-butanol complexes.[343]

Systematic examination of all probable conformations in cellobiose, cellulose, and xylan, assuming ring conformations and the C-1—O—C-4′ bond angle for each pair to be fixed, has led to possible explanations of the known conformational stiffness of cellulose and its solubility properties in alkali.[344] The unperturbed dimensions of cellulose derivatives have been investigated.[345] The perturbed dimensions of cellulose tricarbanilate in acetone, cyclohexanone, and dioxan have been calculated by use of three theories,[346] and those calculated from viscosity data compared well with

[337] S. R. E. Mander, R. M. Purvinas, and H. L. Griffen, *Cereal Chem.*, 1968, **45**, 140.
[338] S. R. Erlander and R. Tobin, *Makromol. Chem.*, 1968, **111**, 194.
[339] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1968, **6**, 241.
[340] W. Banks, C. T. Greenwood, and D. J. Hourston, *Makromol. Chem.*, 1968, **111**, 226.
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[341a] J. Szejtti, M. Richter, and S. Augustat, *Biopolymers*, 1968, **6**, 27.
[342] J. J. Jackobs, R. R. Bumb, and B. Zaslow, *Biopolymers*, 1968, **6**, 1659.
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[345] W. Brown and D. Henley, *Makromol. Chem.*, 1967, **108**, 153.
[346] V. P. Shanbhag, *Arkiv Kemi*, 1968, **29**, 1.

those obtained from light scattering. Determination of the sedimentation coefficient–molecular weight relation for solutions of cellulose tricarbanilate in cyclohexanone indicated that the factors responsible for the intrinsic viscosity and sedimentation coefficient were different.[347] Unperturbed dimensions for this molecule have been obtained from data for good solvents,[348] and from measurements in θ-solvents,[349] consistent with a rather flexible molecule. Evidence has been furnished favouring a triclinic space group with unidirectional chains for both cellulose I and cellulose II.[349a]

E.s.r. spectra have been obtained for the sodium hydroxide–cellulose system which suggested that the signal-generating species was trapped in an alkaline matrix rather than on the pyranoside ring.[350]

A stable β-cyclodextrin hydrate, $C_{42}H_{70}I_{35},(12{\cdot}0 \pm 0{\cdot}5)H_2O$ has been prepared [351] and its water content and enthalpy of solution in water determined in the range 15–30°. N.m.r. and i.r. spectroscopy have been used to investigate the conformation of *O*-methylated amylose and cyclodextrins.[352] C-1- and C-2-Hydrogen atoms in α-(1 → 4)-oligo- and poly-saccharides containing D-glucopyranosyl units were found to be equatorial and axial respectively, consistent with a C-1-conformation. Partially methylated compounds in nonpolar (chloroform, carbon tetrachloride) and polar solvents (DMSO) were studied for hydrogen bonding. The hydroxy-substituent at C-3 of 2,6-di-*O*-methyl-α(or β)-cyclodextrin was found to be intramolecularly hydrogen bonded to the oxygen atom of the methoxy-group at C-2 of the adjacent unit. This hydrogen bond was solvent- and concentration-independent and accounted for the resistance to methylation of C-3-hydroxy-groups in cyclodextrins and probably also amylose. Inclusion compounds of cyclodextrins have been studied.[352a]

The molecular weight of agarose was found to be *ca.* 120,000,[353] and that of agaropectin *ca.* 12,600, by measurement of diffusion coefficient, sedimentation coefficient, and intrinsic viscosity. Agarose solutions underwent rapid oxidation, and were degraded to a level where the ability to gel disappeared, on heating in the presence of air but in the absence of buffering salts.

Investigation of the physico-chemical characteristics of the levan produced by *Streptococcus salivarius* revealed [354] a compact and symmetrical molecule of molecular weight $16–23 \times 10^6$. Electron microscopy showed a spherical or ellipsoidal molecule of axial ratio between 1 and 5, but usually <2. It

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[349] V. P. Shanbhag, *Arkiv Kemi*, 1968, **29**, 163.
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[354] E. Newburn and S. Baker, *Carbohydrate Res.*, 1968, **6**, 165.

was suggested that the levan functioned as a reservoir of substrate for bacterial metabolism within the plaque, being slow to diffuse out. Light scattering and end-group analysis indicated that onuphic acid had $\bar{M}_n$ $1{\cdot}2 \times 10^5$ and $\bar{M}_w$ $2{\cdot}0 \times 10^6$, whilst the radius of gyration was 1250 Å in 1M-potassium chloride and 4330 Å in water.[355]

Changes in a number of the physical properties of polygalacturonic acid paralleled its titration curve (*e.g.* metachromatic shift on binding Ruthenium Red, o.r.d. spectra, and viscosity properties).[356] The results were interpreted as indicating a pH-dependent transition in the conformation of polygalacturonic acid associated with ionisation of the carboxylic acid functional groups.

The native glycogen from rat liver had a molecular weight of 300×10^6 and an average diameter of 1700 Å as determined by light scattering measurements.[357] The chitin fibres of the diatom *Thalassiosina fluvialilis*, Hustedt, have been studied by *X*-ray diffraction and electron microscopy.[358] Investigation of the viscoelastic properties of hyaluronic acid solutions with respect to changes in ionic strength, hydrogen-ion concentration, and polymer concentration indicated that the hyaluronic acid molecule was considerably stiffer at pH 2·5 than at other pH values.[359]

Phase equilibria in dextran–barium hydroxide solutions have been investigated.[360]

[355] F. G. E. Pautard and H. Zola, *Biopolymers*, 1968, **6**, 629.
[356] R. W. Stoddart and K. F. Tipton, *Biochem. J.*, 1968, **109**, 21P.
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[358] N. E. Dweltz, J. R. Colvin, and A. G. McInnes, *Canad. J. Chem.*, 1968, **46**, 1513.
[359] D. A. Gibbs, E. W. Merrill, K. A. Smith, and E. A. Balazs, *Biopolymers*, 1968, **6**, 777.
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9
Glycolipids

Bacterial Glycolipids

The major carbohydrate component from alkaline hydrolysates of the glycolipids of *Streptococcus faecalis* and *S. lactis* was identified as 1-[*O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]glycerol.[361] A second component was tentatively identified as 1-(*O*-α-D-glucopyranosyl)glycerol. Both glycosides were components of the neutral fat and phosphatide fractions. This was the first description of glycosylglycerol phosphatides in bacteria. Several species of Gram-positive and one species of Gram-negative bacteria were shown to contain acylated sugars in their glycolipids.[362] Thus, alkaline hydrolysis of the glycolipids liberated lauric and acetic acids. The glycolipid fraction from *Mycoplasma laidlawii* strain B was shown to contain 1-(*O*-α-D-glucopyranosyl)-2,3-diacyl-D-glycerol and 1-[*O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-glucopyranosyl]-2,3-diacyl-D-glycerol.[363] The phospholipid fraction from the same source consisted of phosphatidyl glucose and phosphatidyl glycerol.

The principal lipids in the cells of *Pseudomonas diminuta* were shown to be glycosyl glycerides and at least three similar glycolipids containing glucose and/or uronic acid.[364] 1-(*O*-β-D-Glucopyranosyl)-2,3-diglyceride and 1-(*O*-β-D-glucopyranosyl uronic acid)-2,3-diglyceride were detected in *P. rubescens*.[365]

A dimannosyl diglyceride, tentatively identified as 1-[*O*-α-D-mannopyranosyl-(1 → 3)-*O*-α-D-mannopyranosyl]-D-glycerol, was obtained from the glycolipid fraction of *Microbacterium lactium*.[366] Alkaline hydrolysis of the glycolipids of *Lactobacillus caseii* ATCC 7479 liberated 1-[*O*-α-D-galactopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]glycerol as the major component and 1-[*O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-galactopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]glycerol as a minor component.[367]

[361] W. Fischer and W. Seyferth, *Z. physiol. Chem.*, 1968, **349**, 1662.
[362] K. Welsh, N. Shaw, and J. Baddiley, *Biochem. J.*, 1968, **107**, 313.
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[364] S. G. Wilkinson, *Biochim. Biophys. Acta*, 1968, **152**, 227.
[365] S. G. Wilkinson, *Biochim. Biophys. Acta*, 1968, **164**, 148.
[366] N. Shaw, *Biochim. Biophys. Acta*, 1968, **152**, 427.
[367] N. Shaw, K. Heatherington, and J. Baddiley, *Biochem. J.*, 1968, **107**, 491.

The structure of a phospholipid from *Bacillus megaterium* has been confirmed as 1,2-diacylgylcerol-3-phosphoryl-1′-[2′-*O*-(2″-amino-2″-deoxy-β-D-glucopyranosyl)]glycerol by total synthesis.[368]

Glycolipids containing galactose, arabinose, and fucose have been found in starfish and sponge,[369] and sphingoglycolipids containing glucose and sialic acid have been isolated from sea-urchin gonads.[370]

Gangliosides

The structure and function of gangliosides has been the subject of a review.[370a] Further evidence was provided for the presence in calf brain of enzymes which catalysed the total, stepwise hydrolysis of di- and trisialogangliosides to sphingosine, fatty acid, and sugars.[371, 371a] Enzymes present in the brains of young rats were shown to catalyse the transfer of ^{35}S-sulphate from ^{35}S-labelled 3′-phosphoadenosine 5′-phosphosulphate to galactose-containing glycosphingolipids.[372] Two glycosyl transferases detected in embryonic chick brain catalysed the following reactions:[373]

$$\text{UDP-G} + \text{ceramide} \rightarrow \text{ceramide-G} + \text{UDP}$$
$$\text{UDP-Gal} + \text{ceramide G} \rightarrow \text{ceramide-G-Gal} + \text{UDP}$$

Other enzymes which catalysed the transfer of sialic acid from CMP-*N*-acetylneuraminic acid to galactosylglucosyl ceramide with the formation of monosialogangliosides were also identified from the same source.[374] Subsequently it was shown [374a] that two monosialogangliosides served as acceptors in similar reactions leading to the formation of disialogangliosides. In one of these reactions, which produced the major disialoganglioside of human brain, sialic acid was transferred to the terminal galactose residue of a ceramide pentasaccharide (80). The other reaction involved the synthesis of (81) in which *N*-acetylneuraminic acid was linked at C-8 to the *N*-glycollylneuraminic acid residue.

Accumulation of G_{M1}-ganglioside (80) and a deficiency of β-galactosidase have been reported [375] in a case of G_{M1}-gangliosidis (Landing disease).

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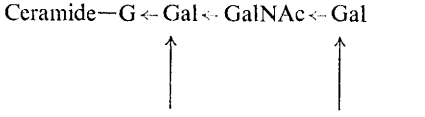

(80)

Ceramide—G ← Gal ← *N*-Glycollyl NA ← NAcNA

(81)

Miscellaneous Glycolipids

1-[*O*-(6-*O*-Acyl-β-D-galactopyranosyl)]-2,3-di-*O*-acyl-D-glyceritols and phytosteryl 6-*O*-acyl-β-D-glucopyranosides have been isolated from soft wheat flour.[375a] Ceramide glucoside, ceramide galactoside, ceramide galactoside sulphate, ceramide lactoside, ceramide digalactoside, 2-acetamido-2-deoxygalactosylglucosyl ceramide, sialylgalactosylglucosyl-ceramide and two glycolipids, one of which contained glucose, galactose, 2-acetamido-2-deoxyhexose (2-acetamido-2-deoxyglucose:2-acetamido-2-deoxyglucose 1 : 1) fucose and ceramide (molar ratios 1 : 2 : 1 : 1 : 1), the other, glucose, galactose, 2-acetamido-2-deoxyglucose, focose, and ceramide (molar ratios 1 : 2 : 2 : 1 : 1) have been isolated from porcine intestine.[375b]

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