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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 3

A Review of the Literature Published during 1969

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Preface

In this, the third Volume of the Series, our aim has been to cover the literature available to us between mid-January 1969 and mid-January 1970. Our overall emphasis is the same as that described in the Preface to Volume 1. Again, *Abstracts of the American Chemical Society Meetings*, *Dissertation Abstracts*, and the patent literature have not been abstracted.

To aid literature searching an index of trivial names of substances covering Volumes 1—3 is included in this Volume.

No recognised abbreviation is used for 'benzyl', though some authors we find use 'Bz' when it should be reserved for 'benzoyl'. We feel there is a need for some recognised abbreviation for the benzyl group and in this Volume we have introduced the use of the abbreviation 'Bn' and hope it may be generally adopted.

We thank Professor N. K. Kochetkov for providing us with English abstracts of a large number of Russian papers.

R. D. G.
R. J. F.
T. D. I.
P. J. S.

March 1970.

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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
Bn	benzyl
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	hexamethylphosphortriamide
i.r.	infrared
LAH	lithium aluminium hydride
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
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
T. D. Inch

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 previous schemes.

Mass spectrometry and *X*-ray diffraction analysis continue to be used increasingly in structural studies. This year was notable for the almost exclusive use of c.d. and o.r.d. in polarimetric investigations. Proton magnetic resonance spectroscopy is now, of course, almost obligatory in structural investigations, and several papers this year on ^{13}C n.m.r., especially on unenriched samples, have perhaps indicated an area for major development in the near future. The nucleoside antibiotic, nucleocidin, first reported in 1957, has now had its structure firmly established, and has been shown to be the first naturally occurring derivative of a fluoro-carbohydrate. The first sugar analogues with phosphorus as the ring hetero-atom have been synthesised, and the controversy over the structure of sugar osazones has been reopened. Important reports on glycosyl derivatives have appeared from Lemieux's and from Fletcher's laboratories. The former group have investigated solvation phenomena, and particularly their influence on the anomeric effect, whilst the latter have made observations on solvolysis reactions of glycosyl halides that could lead to improved methods for the synthesis of α-glucosides.

Apart from Volume 2 of this series, other books and reviews of general interest appeared during 1969, including the regular report in the Chemical Society's Annual Reports,[1] and *Advances in Carbohydrate Chemistry*, volume 24. A review of recent developments in the chemistry of monosaccharides,[2] and one on configurational analysis in carbohydrate chemistry [3] have been written by Ferrier. A review on mechanism in carbohydrate chemistry has appeared, covering all classes of monosaccharide derivatives.[3a] The basic rules of carbohydrate chemistry have been described.[4] An obituary of Richard Kuhn has appeared,[4a] as has an

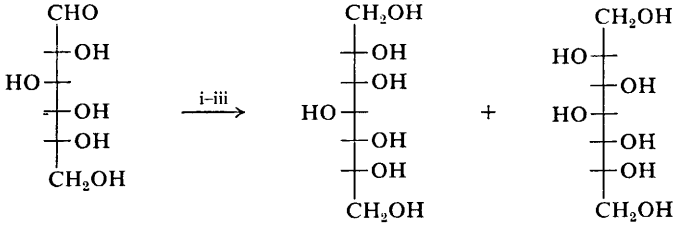
[1] *Ann. Reports* (*B*), 1968, **65**, 441.
[2] R. J. Ferrier, *Chem. in Britain*, 1969, **5**, 15.
[3] R. J. Ferrier, 'Progress in Stereochemistry,' vol. 4, ed. B. J. Aylett and M. M. Harris, Butterworth, London, 1969, p. 43.
[3a] B. Capon, *Chem. Rev.*, 1969, **69**, 407.
[4] S. T. Reid, *Pharm. J.*, 1969, **202**, 733.
[4a] H. H. Baer, *Adv. Carbohydrate Chem.*, 1969, **24**, 1.

appreciation of the scientific career of Max Bergmann on the twenty-fifth anniversary of his death,[5] and a special number of *Carbohydrate Research* [5a] was issued in memory of Professor Stanley Peat.

[5] B. Helferich, *Chem. Ber.*, 1969, **102**, I.
[5a] *Carbohydrate Research*, April, 1969.

2
Free Sugars

A system based on 'stereonumbers' has been described as an aid to the easy learning of hexose configurations.[6] A second report has suggested[7] that for teaching purposes the Fischer proof of the relative configuration of the asymmetric atoms in glucose is too complex for the beginning student, and that an easier concept for the differentiation of glucose and mannose in the final stage is the sequence shown for glucose in Scheme 1. From a similar sequence mannose affords two optically active heptitols.



meso optically active

Reagents: i, MeONa, $MeNO_2$; ii, H^+; iii, H_2–Ni

Scheme 1

The interesting and somewhat unexpected observation has been made that enantiomeric sugars have the same sweetness[8] (see Table 1).

Table 1

Sugar	*Sweetness score*	
	D-	L-
Arabinose	5·2	5·6
Xylose	4·6	4·4
Glucose	5·4	6·0
Rhamnose	4·6	6·5
Mannose	4·9	5·0
Galactose	5·6	6·0
Glucoheptose	5·2	6·6
Fructose	Very sweet	Very sweet

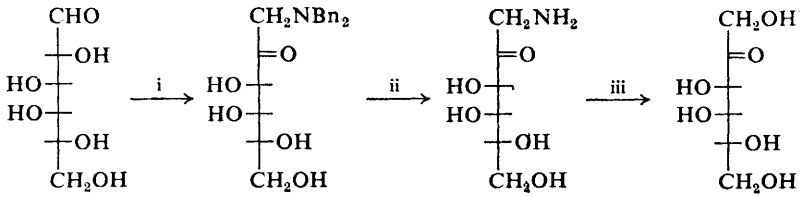
[6] S. Neelakantan, *Current Sci.*, 1969, **38**, 353.
[7] Y. Z. Frohwein, *J. Chem. Educ.*, 1969, **46**, 55.
[8] R. S. Shallenberger, T. E. Acree, and C. Y. Lee, *Nature*, 1969, **221**, 555.

A structural hypothesis to explain sweetness has been proposed which indicates how sweetness varies with configuration and ring conformation.[9]

Isolation anp Synthesis

The main free carbohydrates present in grass pollens have been identified as D-fructose, D-glucose, and *myo*-inositol.[10] L-Gulose and 3-*O*-carbamoyl-D-mannose have been found in natural products for the first time as components of the antibiotic bleomycin A2.[11]

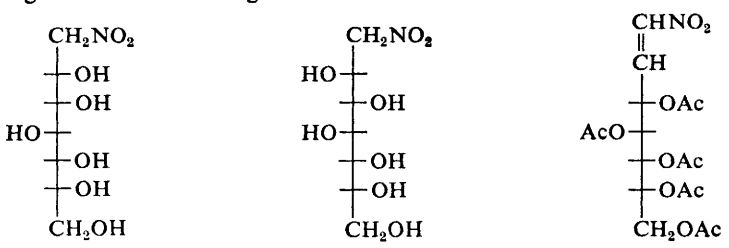
Full details have been published [12] of the synthesis of coriose (D-*altro*-3-heptulose) (see vol. 2, p. 4). D-Tagatose has been prepared from D-galactose in 16% overall yield (Scheme 2).[13]



Reagents: i, Bn_2NH; ii, H_2–Pd; iii, HNO_2

Scheme 2

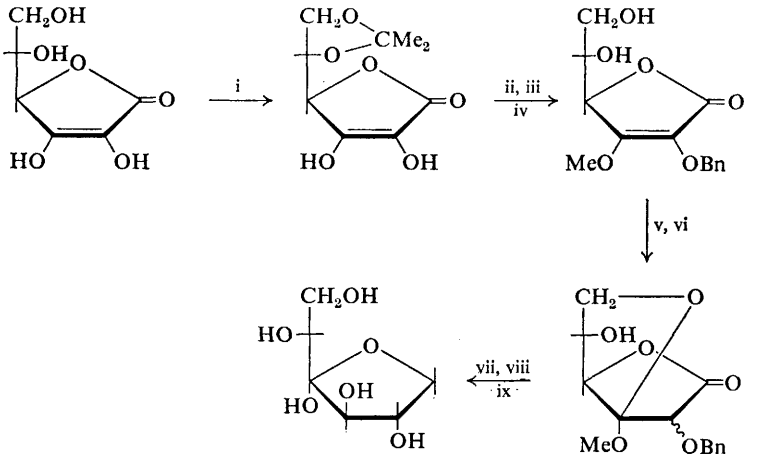
D-*glycero*-D-*gulo*-Heptose and D-*glycero*-D-*ido*-heptose have been prepared in 11 and 9% yields respectively by fractional crystallisation of the nitrosugars (1) and (2) formed by condensation of D-glucose and nitromethane.[14] It is claimed that the procedure used is practically significant, since previously, similar experiments gave inadequate yields. Both (1) and (2) were converted into (3) which afforded 2-deoxy-D-*gluco*-heptose on hydrogenation and Nef degradation.



(1) (2) (3)

[9] R. S. Shallenberger and T. E. Acree, *J. Agric. Food Chem.*, 1969, **17**, 701.
[10] E. J. Shellard and G. H. Jolliffe, *J. Chromatog.*, 1969, **40**, 458.
[11] T. Takita, K. Maeda, H. Umezawa, S. Omoto, and S. Umezawa, *J. Antibiotics*, 1969, **22**, 237.
[12] T. Okuda and K. Konishi, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 735.
[13] R. Grünnagel and J. H. Haas, *Annalen*, 1969, **721**, 234.
[14] D. T. Williams and M. B. Perry, *Canad. J. Chem.*, 1969, **47**, 2763.

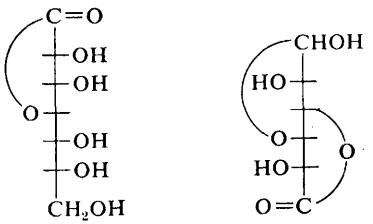
The synthesis of L-*xylo*-3-hexulose from L-ascorbic acid has been achieved (Scheme 3).[15]



Reagents: i, Me_2CO–H^+; ii, CH_2N_2; iii, Na, BnCl; iv, H^+; v, NaOH; vi, H^+; vii, LAH; viii, H_2–Ni; ix, isolation and acid hydrolysis

Scheme 3

A synthesis of L-glucuronic acid has been described, and the acid has been reduced to L-glucose.[16] The processes involved represent a conversion of D-glucose into the L-enantiomer. Oxidation of D-*glycero*-D-*gulo*-heptonolactone (4) (obtained from D-glucose by Kiliani synthesis) with an equimolar quantity of periodic acid gave D-arabinose and L-glucurone (5) by oxidations at positions 2,3 and 6,7 respectively. L-Glucurone was

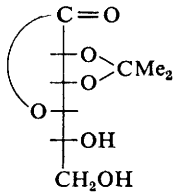


(4) (5)

reduced to L-glucose by way of the 1,2-*O*-isopropylidene acetal using sodium borohydride in the presence of boric acid.

L-Erythrose has been synthesised from the commercially available 2,3-*O*-isopropylidene-D-gulono-1,4-lactone (6) by sequential borohydride

[15] K. G. A. Jackson and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 2498.
[16] W. Sowa, *Canad. J. Chem.*, 1969, **47**, 3931.



(6)

reduction, periodate oxidation, and acidic hydrolysis.[17] D-Arabinose was formed by irradiation with a mercury lamp for 3 h of 2-deoxy-2-(2,4-dinitroanilino)-D-gluconic acid.[18]

Modified procedures have been described for the separation of D-*threo*-pentulose and D-*erythro*-pentulose (as their acetals) formed by pyridine-induced isomerisations of D-xylose and D-arabinose.[19]

[^{3}H]Lithium aluminium hydride reduction of sodium D-*arabino*-hexul-2-osonate afforded [2-^{3}H]D-glucose and [2-^{3}H]D-mannose derivatives in equivalent yields.[20] Reduction of the derived lactones with sodium amalgam gave the free sugars which lost tritium on conversion into phenylosazones. Enzymic methods have been used for the synthesis of ^{14}C-3 and ^{14}C-4 labelled D-glucose.[21] Thus the enzymic carboxylation of D-*erythro*-pentulose 1,5-diphosphate with $^{14}CO_2$ gave phosphoglyceric acid [1-^{14}C]-3-phosphate which by reversal of glycolytic processes may serve as a source of 3,4-labelled glucose. For the synthesis of ^{14}C-3 labelled hexoses, labelled dihydroxyacetone phosphate can be used; ^{14}C-4-labelled isomers can be derived from labelled glyceric acid phosphate and unlabelled dihydroxyacetone phosphate.

A review has appeared (in Russian) on the synthesis of carbohydrates from formaldehyde.[22]

Physical Measurements

The second part of a two-part review (see also vol. 2, p. 5) on the mutarotation of sugars in solution has appeared,[22a] which covers catalytic processes, isotope effects, reaction mechanisms, and biochemical aspects.

Modern concepts and techniques continue to be applied in studies of the mutarotation of sugars. The rate constants (obtained by optical rotation measurements and by gas chromatographic analysis of trimethylsilylated mixtures) for the mutarotations of D-glucose and D-mannose were in good

[17] L. M. Lerner, *Carbohydrate Res.*, 1969, **9**, 1.
[18] A. E. El. Ashmawy, D. Horton, and K. D. Philips, *Carbohydrate Res.*, 1969, **9**, 353.
[19] R. S. Tipson and R. F. Brady jun., *Carbohydrate Res.*, 1969, **10**, 549.
[20] H. S. Isbell, H. L. Frush, C. W. R. Wade, and A. J. Fatiadi, *J. Res., Nat. Bur. Stand.*, 1969, 73A, 75.
[21] E. Sturani, *J. Labelled Compounds*, 1969, **5**, 47.
[22] I. L. Orestov, *Vop. Istor. Estestvozn. Tekh.*, 1968, 56 (*Chem. Abs.*, 1969, **70**, 29,210z).
[22a] H. S. Isbell and W. Pigman, *Adv. Carbohydrate Chem.*, 1969, **24**, 14.

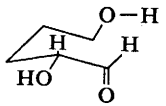
agreement,[23] and there was no evidence for participation by furanose forms. However, the thermodynamic data from the equilibrium methods were not in good agreement with those obtained by other techniques, suggesting that the structure of the transition state is significantly different from either pyranose anomer. Similar kinetic analysis of the mutarotation of α-D-galactopyranose has been carried out.[24] The initial rapid stage of mutarotation was due to the formation of α-D-galactofuranose and β-D-galactofuranose; the slow stage, to formation of β-D-galactopyranose. A detailed study of the mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucose in benzene and benzene–methanol has been reported.[25] Strong nucleophilic reagents are effective general base catalysts. Acid and base catalysts, *e.g.* mixtures of pyridine and phenols, function as general base catalysts. No evidence for a concerted general acid–base catalysed mechanism was obtained. A second group[26] has also reported on the mutarotation of tetra-*O*-methyl-α-D-glucopyranose catalysed by organic bases. The mutarotation of α- and β-D-glucose in pyridine has been followed by gas chromatography.[27] Kinetic analysis has indicated that the mutarotation was autocatalytic and that a sterically acceptable mechanism for the reaction was a modification of that due to Swain and Brown. The mutarotation of monosaccharides in solution has been investigated[28] by deep-freezing in liquid air at various time intervals, followed by lyophilisation, silylation, and gas chromatography. By this procedure, mutarotated fructose was found to be a five-component mixture of the α- and β-pyranose, α- and β-furanose, and open-chain forms.

The kinetics of the mutarotation of D-glucose catalysed by α-amino acids and amino-carboxylic acid mixtures have been studied,[29] and a preliminary report of the kinetics of the mutarotation of tetra-*O*-methyl-α-D-glucose catalysed by the asymmetric naphthoxypropionic acids has appeared.[30] The stereoisomers of α-phenylethylamine and *threo*-2-amino-1-(*p*-nitrophenyl)propan-1,3-diol have been studied as catalysts for the mutarotation of α-L-rhamnose.[31] In the former case the (−)-enantiomer was more active and explanations to account for this stereoselectivity were proposed. The same group of workers[32] then found that the second catalyst was efficient for catalysing the mutarotation of β-D-arabinose.

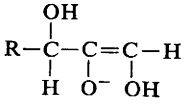
[23] C. Y. Lee, T. E. Acree, and R. S. Shallenberger, *Carbohydrate Res.*, 1969, **9**, 356.
[24] T. E. Acree, R. S. Shallenberger, C. Y. Lee, and J. W. Einset, *Carbohydrate Res.*, 1969, **10**, 355.
[25] P. R. Rony, W. E. McCormack, and S. W. Wunderly, *J. Amer. Chem. Soc.*, 1969, **91**, 4244.
[26] H. H. Huang, A. N. H. Yeo, and L. H. L. Chia, *J. Chem. Soc.* (*C*), 1969, 836.
[27] A. S. Hill and R. S. Shallenberger, *Carbohydrate Res.*, 1969, **11**, 541.
[28] H. C. Curtius, J. A. Voellmin, and M. Mueller, *Z. analyt. Chem.*, 1968, **243**, 341.
[29] V. A. Afanas'ev and N. I. Trushkina, *Khim. Fiz.-Khim. Uglevodov*, 1968, 3 (*Chem. Abs.*, 1969, **71**, 81,678*t*).
[30] A. Kergomard and M. Renard, *Tetrahedron Letters*, 1969, 3041.
[31] V. A. Paulov, E. I. Klabunovskii, and A. A. Balandin, *Zhur. fiz. Khim.*, 1968, **42**, 2475.
[32] V. A. Paulov, E. I. Klabunovskii, and A. A. Balandin, *Zhur. fiz. Khim.*, 1968, **42**, 2481.

Again stereoselective effects were noted. Effects of temperature have also been studied.[33]

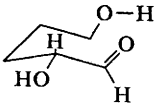
The rate of tritium uptake by glucose in alkaline tritiated water has been shown to be 1000 times less than for two inoses. This low rate of reaction suggests that the rate of enolisation depends on an intermediate carbonyl form of sugar.[34] To account for well-known differences in mutarotation and epimerisation of sugars, it was suggested that mutarotation proceeds through pseudo acyclic intermediates that possess certain characteristics of the parent ring forms. This new concept applied to the interpretation of enolisation reactions leads to the possibility of the formation of *cis*- and *trans*-enediols, the proportions of which vary from sugar to sugar, as shown in Scheme 4.



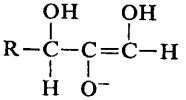
from α-D-glucopyranose



cis-ene-diol



from β-D-glucopyranose



trans-ene-diol

Scheme 4

Various other kinetic studies of reactions of free sugars have been reported. The kinetics of the conversion of the pentoses into furfural have been studied and mechanisms for the reactions proposed.[35] The order of reactivities was ribose > xylose > lyxose > arabinose. Kinetic measurements have been reported for the oxidation of glucose with ammoniacal silver nitrate,[36] and for the oxidation of glucose and fructose with potassium ferricyanide.[37] The rates of enzymic and acid-catalysed hydrolyses of sucrose have been compared and found to be quite different at low temperatures.[38] In the latter case an Arrhenius plot was linear between 12 and −7 °C, but below this temperature this relationship was no longer obeyed and a maximum was unexpectedly found near −12 °C. Rates of interconversion of D-glucose, D-mannose, and D-fructose in M-NaOH at 22 °C have been measured by neutralising aliquot samples, resolving the components on anion resin columns, and applying automatic colorimetric

[33] V. A. Paulov, E. I. Klabunovskii, and A. A. Balandin, *Zhur. fiz. Khim.*, 1968, **42**, 2487.
[34] H. S. Isbell, H. L. Frush, C. W. R. Wade, and C. E. Hunter, *Carbohydrate Res.*, 1969, **9**, 163.
[35] E. R. Garrett and B. H. Dvorchik, *J. Pharm. Sci.*, 1969, **58**, 813.
[36] A. P. Modi and S. Ghosh, *J. Indian Chem. Soc.*, 1969, **46**, 687.
[37] K. C. Gupta and M. P. Singh, *Bull. Chem. Soc. Japan*, 1969, **42**, 607.
[38] D. B. Lund, and O. Fennema, W. D. Powrie, *J. Food Sci.*, 1969, **34**, 378.

analytical procedures.[39] The isomerisation of glucose to mannose was noticeably slower than the other reactions, and the glucose to fructose reaction was particularly facile. Reasons for these observations were discussed. D-Allose and D-altrose could not be detected in the products. Unspecified degradation reactions were also observed and assessed kinetically.

Other physical data reported have included information concerning rotational changes which occurred when α-D-glucopyranose was heated,[40] activity coefficients of solutions of maltose and xylose,[41] and diffusion coefficients of solutions of maltose and xylose.[42]

Free sugars have been subjected to field ionisation mass spectrometry. The M^+ and $(M+1)^+$ ions were clearly visible, and the technique may be useful in molecular weight determinations of unsubstituted sugars.[43]

Detailed o.r.d. and c.d. studies of molybdate–pyranose complexes have been reported.[44]

An important review [45] has appeared on the conformations and equilibrium positions adopted by aldoses in solution.

Reactions

The electrolysis of carbohydrates has been reviewed, and the electrolysis of monosaccharides in alkaline non-aqueous media described.[46] Free aldoses were degraded sequentially to lower aldoses without the formation of detectable acidic products, and maltose underwent cleavage of the glycosidic bond.

A review [47] has been published on the effects of heat on sugars and polysaccharides. Mechanisms of reactions which lead to volatile and non-volatile products were discussed, and possible relationships between thermal degradation products of carbohydrates and compounds found in foodstuffs were pointed out. Self-condensation of the common hexoses at 200 °C has been followed by thermogravimetric procedures [48] (see vol. 1, p. 10). The order of reactivity was found to be α-D-galactose > β-D-galactose > α-D-mannose > β-D-mannose > α-D-glucose > β-D-glucose. The rates of isomerisation and degradation of cellobiose, cellobiulose and 4-*O*-β-D-glucopyranosyl-D-mannose were determined in 1M-NaOH at 22 °C as for the corresponding hexoses.[49] All the reactions were discussed

[39] D. J. MacLaurin and J. W. Green, *Canad. J. Chem.*, 1969, **47**, 3947.
[40] N. Nath and V. N. Singh, *Indian J. Chem.*, 1969, **7**, 329.
[41] H. Uedaira and H. Uedaira, *Bull. Chem. Soc. Japan*, 1969, **42**, 2137.
[42] H. Uedaira and H. Uedaira, *Bull. Chem. Soc. Japan*, 1969, **42**, 2140.
[43] H. Krone and H. D. Beckey, *Org. Mass Spectrometry*, 1969, **2**, 427; H. D. Beckey, *Angew. Chem. Internat. Edn.*, 1969, **8**, 623.
[44] W. Voelter, E. Bayer, R. Records, E. Bunneberg, and C. Djerassi, *Annalen*, 1968, **718**, 238.
[45] S. J. Angyal, *Angew. Chem. Internat. Edn.*, 1969, **8**, 157.
[46] G. W. Hay and F. Smith, *Canad. J. Chem.*, 1969, **47**, 417.
[47] I. S. Fagerson, *J. Agric. Food Chem.*, 1969, **17**, 747.
[48] J. W. Liskowitz and B. Carroll, *J. Macromol. Sci.*, 1968, **2**, 1139.
[49] D. J. MacLaurin and J. W. Green, *Canad. J. Chem.*, 1969, **47**, 3957.

in detail and, for example, the breakdown of cellobiose to glucose and the subsequent isomerisation to other hexoses was discussed.

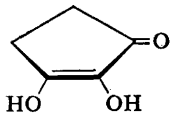
A quick and convenient method for the degradation of labelled sugars in order to assess activities at individual positions has been described.[50] The quantitative enzymic conversion of D-glucose into lactic acid, followed by chemical degradation of the acid, allowed discrimination between C-1 and C-6, C-2 and C-5, and C-3 and C-4. The enzymic conversion into glycerol-1-phosphate and glyceric acid 3-phosphate allowed the distinction to be made between C-1, 2, 3 and C-4, 5, 6. A study of the direct photolysis of D-glucose in aqueous solution has shown by the use of ^{14}C-1-labelled material that all chemical changes result from excitations at and subsequent dissociations near the ring oxygen atom.[51] Some radicals formed upon radiolysis of frozen aqueous solutions of ribose have been identified by e.s.r. spectroscopy and a mechanism for their formation has been suggested.[52] The important observation has been made that epimerisation of monosaccharides took place on radiolysis of frozen aqueous solutions of D-pentoses. Thus, D-arabinose, L-lyxose, and xylose were formed from D-ribose and only lyxose from D-arabinose and D-xylose. Arabinose was formed from lyxose. Mechanisms were suggested for these reactions.[53]

The reactions of carbohydrates and their derivatives with hydrogen fluoride have been reviewed.[54] Reaction of D-glucose with anhydrous hydrogen fluoride caused polymerisation mainly by the formation of 1,6-α linkages.[55] The acid stabilities of the common hexoses have been determined by gas chromatographic methods.[55a] The action of sodium hypochlorite on many sugars in aqueous borate and carbonate buffers has been studied,[56] and a thermochemical study of the oxidation of glucose with hypochlorous acid and sodium hypochlorite has been reported.[57] The relative reducing powers of sugars with respect to chromate have been investigated and the results interpreted on a structural basis.[58] The order of reducing power was glucose > galactose > mannose and ribose > xylose > arabinose > lyxose. It has been observed that green fluorescent substances are formed during the conversion of fructose to 5-hydroxymethylfurfural, and that sucrose, raffinose, and sorbose also provide these

[50] E. Sturani and S. Cocucci, *J. Labelled Compounds*, 1969, **5**, 42.
[51] G. O. Phillips and T. Rickards, *J. Chem. Soc.* (C), 1969, 455.
[52] N. K. Kochetkov, L. I. Kudriashov, M. A. Chlenov, V. A. Sharpatii, M. T. Nadgymiddinova, I. V. Nikitin, and N. M. Emanuel, *Doklady Akad. Nauk S.S.S.R.*, 1968, **183**, 376.
[53] N. K. Kochetkov, L. I. Kudriashov, M. A. Chlenov, and T. Ya. Livertovskya, *Doklady Akad. Nauk S.S.S.R.*, 1969, **187**, 332.
[54] J. Lenard, *Chem. Rev.*, 1969, **69**, 625.
[55] T. Hanada and M. Yoshida, *J. Chem. Soc. Japan*, 1969, **90**, 201.
[55a] Y. Nozawa, Y. Hiraguri, and Y. Ito, *J. Chromatog.*, 1969, **45**, 244.
[56] Z. A. Maslinkovskaya, N. Ya. Kuznetsova-Lenshina, and V. Ivanov, *Khim. Fiz.-Khim. Uglevodov*, 1968, **21** (*Chem. Abs.*, 1969, **71**, 91,786*r*).
[57] A. E. Bernardelli, T. T. Grigor, and T. A. Tumanova, *Zhur. priklad. Khim.*, 1968, **41**, 2105.
[58] M. N. Tul'Chinskii, *Izvest. V. U. Z., Khim. i khim. Tekhnol.*, 1968, **11**, 556 (*Chem. Abs.*, 1968, **69**, 97,033*y*).

substances.[59] It was pointed out that glucose, maltose, and lactose do not produce these compounds and that those sugars giving them contain furanoid structures. A study was made [60] of the Maillard reaction occurring when glucose was heated in water containing 2-aminoethanol. Fluorescent products were obtained, examined, and surprisingly identified as flavanoid glycosides. The formation of alkylated pyrazines from amino-acids and carbohydrates is believed to give rise to the 'roasted nut' smell associated with several foodstuffs.[61] It was shown by use of radioisotope labelling studies that the carbon atoms of these products were derived from sugars whereas nitrogen was furnished by the amino-acids.

Reductic acid (6A) formed by acidic degradation of [1-^{14}C]D-xylose had the same ^{14}C distribution as (6A) formed from 2-[α-^{14}C]furfuraldehyde, thus suggesting that 2-furfuraldehyde was an intermediate in the degradation of D-xylose.[62]



(6A)

The mechanism of epimerisation of cellobiose and 4-*O*-β-D-glucopyranosyl-D-mannose by enzymes of *Ruminococcus albus* has been investigated.[63] Only the proton of C-2 was exchanged during the process and cellobiulose was not isomerised. The involvement of a C-2 carbanion was suggested.

[59] J. Hashimoto, *J. Chem. Soc. Japan*, 1968, **89**, 1266; 1969, **90**, 320, 920.
[60] Y. Campagne and H. Margulis, *Ind. Aliment. Agr.* (*Paris*), 1969, **86**, 501 (*Chem. Abs.*, 1969, **71**, 102,140*d*).
[61] P. E. Koehler, M. E. Mason, and J. A. Newell, *J. Agric. Food Chem.*, 1969, **17**, 393.
[62] J. J. Schneider and N. S. Bhacca, *J. Org. Chem.*, 1969, **34**, 1990.
[63] M. Amein and J. M. Leatherwood, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 223.

3
Glycosides

O-Glycosides

Synthesis.—A few new developments have been reported in this area and many applications of both standard and more recently developed methods have been described; particularly noteworthy are the use of trimethylsilylated glycosyl halides (p. 19) and the greater insight into the solvolysis of glycosyl halides which Ishikawa and Fletcher have gained.[64] For example, they have shown that the readily accessible 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucosyl bromide and its anomer were both convertible into α-glycosides in high yield. This important work is outlined in greater detail on p. 60. For the Koenigs–Knorr reaction, the potentially valuable observation has been made that the hydroxy-group of the aglycones can be activated by conversion into t-butyl ethers.[65]

Full papers on the synthesis of 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosides and their hydroxylations to give saturated α-glycosides have been published. Related 3-deoxy-hex-2-enopyranosides have also been reported (see p. 111). A method of some general applicability for the synthesis of alkyl α-D-glucopyranosides involved the reaction of penta-*O*-acetyl-β-D-glucopyranose with alcohols in the presence of an acid catalyst. The β-glycosides present in the products were hydrolysed enzymically, and the glucose formed removed either by use of anion-exchange resin or with D-glucose oxidase. Several new glycosides were prepared in this way. Yields >70% were reported.[66] A similar approach was adopted to obtain methyl α-D-[gluco-^{14}C(u)]pyranoside, which was prepared using the free sugar in methanol in the presence of cation-exchange resin. Again the β-anomer produced was removed with almond emulsin.[67]

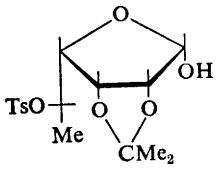
Methanolysis of 2-deoxy-D-*ribo*-hexose under mild conditions gave a quantitative yield of a crystalline product which contained equimolar proportions of the α- and β-furanosides. From this mixture the former can be obtained pure, and, by way of a glycosyl bromide intermediate, the latter was also obtained pure from it. In the course of this investigation

[64] T. Ishikawa and H. G. Fletcher jun., *J. Org. Chem.*, 1969, **34**, 563.
[65] N. K. Kochetkov, V. A. Derevitskaya, and E. M. Klimov, *Tetrahedron Letters*, 1969, 4769.
[66] R. E. Wing and J. N. BeMiller, *Carbohydrate Res.*, 1969, **10**, 441.
[67] G. M. Bartlett and G. Sheppard, *J. Labelled Compounds*, 1969, **5**, 275.

both the crystalline methyl pyranosides were also prepared.[68] An improved method for synthesising methyl 2-deoxy-α-D-*arabino*-hexofuranoside involved mercury-catalysed ring closure in methanol of the dibenzyl or diethyl dithioacetals of 2-deoxy-D-*arabino*-hexose.[69] Methanolysis of penta-*O*-acetyl-α- and -β-D-gluco-, -galacto-, and -manno-pyranoses indicated that furanosides were formed exclusively. Such a result would appear to require that solvolysis occurred more slowly at the anomeric centre than at the other secondary ring positions.[70] Ethanolysis of D-galactose by heating under reflux for 1 h in the presence of Dowex-50 resin gave the ethyl β-furanoside and the two pyranosides. From penta-*O*-acetyl-galactofuranose, the *m*-tolyl and guaiacol β-furanosides were also synthesised. The stability of these β-furanosides towards β-galactosidase was investigated.[71] A synthesis of benzyl 1,3,4,6-tetra-*O*-benzoyl-α-D-fructofuranoside has been reported.[72]

Further work on the acid treatment of 2-*O*-(hydroxyethyl)-D-glucose has corroborated previous findings that a mixture of furanoid and pyranoid intramolecular glucosides is formed (*cf.* vol. 2, p. 14).[73]

Kuhn methylation of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose yielded a 99 : 1 mixture of the α- and β-methyl glycosides. Surprisingly, however, when the sodio-derivative of the sugar was treated with methyl iodide, a 1 : 10 mixture of anomers was obtained. These observations, therefore, provide convenient means for synthesising both methyl D-mannofuranosides.[74] Some insight was gained into this apparent anomaly by the demonstration that, with methyl iodide and silver oxide, compound (7) was



(7)

methylated more rapidly than its α-anomer. This difference was attributed to intramolecular hydrogen bonding which increased the nucleophilic character of the oxygen atom at C-1 of the reactive anomer.[75] The methylation of 2,3-*O*-isopropylidene-L-rhamnose was then studied under both Kuhn and Purdie conditions. In the latter case, a mixture of the anomeric

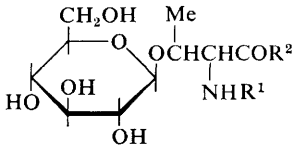
[68] C. C. Bhat, K. V. Bhat, and W. W. Zorbach, *Carbohydrate Res.*, 1969, **10**, 197.
[69] W. W. Zorbach, C. C. Bhat, and K. V. Bhat, *Carbohydrate Res.*, 1969, **11**, 140.
[70] J. Struciński and A. Ksiezopolska, *Roczniki Chem.*, 1969, **43**, 1305.
[71] K. Yoshida, N. Iino, T. Kamata, and K. Kato, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1123.
[72] A. Klemer and B. Dietzel, *Carbohydrate Res.*, 1969, **11**, 285.
[73] E. J. Roberts and S. P. Rowland, *Canad. J. Chem.*, 1969, **47**, 1592.
[74] M. H. Randall, *Carbohydrate Res.*, 1969, **11**, 173.
[75] A. H. Haines, *Tetrahedron Letters*, 1969, 1201.

furanosides and pyranosides was produced with the α-furanoside predominating. In DMF, only the α-furanoside and α-pyranoside were obtained. This result agreed with work done on the mutarotation of the sugar in DMF, which showed that α,β equilibrations were very slow and only the α-furanose ⇌ α-pyranose equilibria need be considered.[76]

The orthoester glycoside synthesis continues to be developed and applied. The study of the condensation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose with acetylated ethyl, isopropyl, t-butyl, and phenyl orthoacetates of α-D-glucopyranose has shown that the t-butyl derivatives are the best glycosylating agents.[77] The butyl orthoesters were then employed for glycosylation of the primary hydroxy-group of gentiobiose hepta-acetate (75% yield), and the secondary groups of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (50%) and 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (25%).[78] This general approach has also been applied to obtain the β-maltoside and β-melibioside of the *N*-methylamide of L-serine,[79] the β-glucopyranosides of β-naphthol, *p*-methoxyphenol, and *p*-hydroxyacetophenone,[80] and of several sterols and triterpenes.[81]

A related method of glycoside synthesis, which involves glycosyl 1,2-acetoxonium ions, has been used in the chemical synthesis of oligosaccharides. Fusion of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose with catalytic amounts of toluene-*p*-sulphonic acid afforded, after deacetylation, 1,6-anhydro-β-D-glucopyranose, gentiobiose, gentiotriose, and higher β-1,6-linked oligosaccharides. The same approach was also applied to obtain 1,2-, 1,3-, and 1,4-linked products.[81a]

The Koenigs–Knorr synthetic method retains its importance in spite of the above developments. The glucosyl L-threonine derivatives (8)—(10)



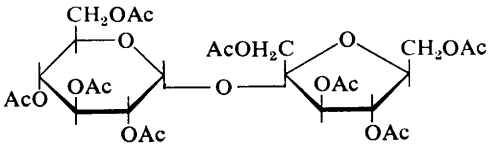
	R^1	R^2
(8)	BnOCO	OMe
(9)	BnOCO	NHMe
(10)	H	NHMe

were prepared using the acylated glycosyl bromide, and galactosyl analogues were synthesised in similar fashion.[82] 2-Amino-2-deoxy-D-*gluco*-com-

[76] A. C. Ferguson and A. H. Haines, *J. Chem. Soc.* (*C*), 1969, 2372.
[77] A. F. Bochkov, T. A. Sokolovskaya, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 1570.
[78] N. K. Kochetkov, A. F. Bochkov, and T. A. Sokolovskaya, *Doklady Akad. Nauk S.S.S.R.*, 1969, **187**, 96.
[79] N. K. Kochetkov, V. A. Derevitskaya, A. M. Likhosherstov, V. M. Kalinevich, and O. S. Novikova, *Izvest. Akad. Nauk S.S.S.R., Ser. Khim.*, 1969, 1109.
[80] A. F. Bochkov, A. Ch. Jain, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1143.
[81] G. B. Elyakov, N. I. Uvarova, I. V. Dardymov, O. E. Mislitskaya, and L. M. Antonik, *Khim.-Farm. Zhur.*, 1969, **3**, 5 (*Chem. Abs.*, 1969, **71**, 102,179*y*).
[81a] D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohydrate Res.*, 1969, **11**, 453, 461.
[82] N. K. Kochetkov, E. M. Klimov, and V. A. Derevitskaya, *Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1969, 2779.

pounds were also obtained in this way, the anisylidene group being used to protect the carbohydrate amino-function.[83] In the synthesis of 3-*O*-α-D-glucopyranosyl-L-serine, 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride was employed together with *N*-benzyloxycarbonyl-L-serine benzyl ester.[84] Cholesteryl β-D-glucuronide was prepared in very high yield using the acetyl-glycosyl α-bromide of the methyl ester of glucuronic acid.[85]

As always, the Koenigs–Knorr reaction has been applied in disaccharide syntheses, and this year non-reducing disaccharides have received more attention. Octa-*O*-acetyl-α-D-glucopyranosyl α-D-fructofuranoside (11)

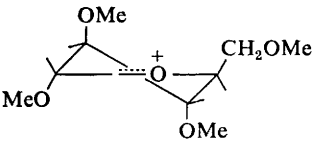


(11)

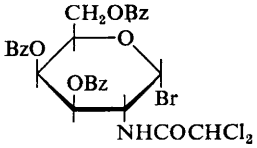
and the corresponding β-D-glucopyranosyl derivative, which are anomers of sucrose, have seen synthesised by the reaction of trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside with 1,3,4,6-tetra-*O*-benzoyl-α-D-fructosyl bromide followed by deacylation and acetylation.[86] The same technique was used to prepare α,α-trehalose derivatives: *e.g.* trimethylsilyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside with 2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl chloride in the presence of silver perchlorate gave the octamethyl ether.[87] In related fashion, α,α- and α,β-trehalose derivatives have been prepared from the reaction of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose with perchloric acid. The key intermediate was believed to be (12).[88] The unsubstituted α,α- and α,β-trehaloses have also been obtained in 18% and 2% yield, respectively, by the reaction of 2,3,4,6-tetra-*O*-benzyl-D-glucose with its glycosyl chloride followed by a hydrogenolysis step.[89] German workers have published an improved method which adopted the same approach.[90]

2-*O*-β-D-Xylopyranosyl-D-glucose was synthesised by use of the mercuric cyanide–mercuric bromide catalyst in acetonitrile, with 2,3,4-tri-*O*-acetyl-α-D-xylosyl bromide and benzyl 3,5,6-tri-*O*-benzyl-α-D-glucofuranoside. The disaccharide is named 'sambubiose' and is found as the carbohydrate component of a naturally occurring glycoside.[91] The synthesis of 5-*O*-(α-D-glucopyranosyl)-D-glucose is mentioned on p. 24.

[83] V. A. Derevitskaya, E. M. Klimov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 966.
[84] K. Kum, *Carbohydrate Res.*, 1969, **11**, 269.
[85] M. S. Feather, *J. Org. Chem.*, 1969, **34**, 1998.
[86] A. Klemer, K. Gaupp, and E. Buhe, *Tetrahedron Letters*, 1969, 4585.
[87] A. Klemer and E. Buhe, *Tetrahedron Letters*, 1969, 1689.
[88] A. Klemer and R. Kutz, *Tetrahedron Letters*, 1969, 1693.
[89] G. J. F. Chittenden, *Carbohydrate Res.*, 1969, **9**, 323.
[90] F. Micheel, and E. D. Pick, *Tetrahedron Letters*, 1969, 1695.
[91] B. Erbing and B. Lindberg, *Acta Chem. Scand.*, 1969, **23**, 2213.



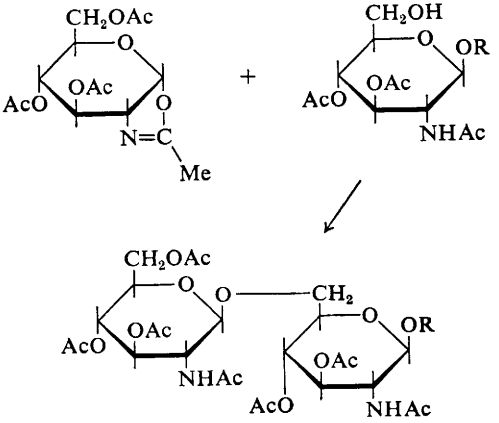
(12)



(13)

An aminodisaccharide having the amino-function in the reducing moiety, 2-acetamido-2-deoxy-6-(*O*-β-L-fucopyranosyl)-D-glucose, has been prepared by condensation of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide with benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranoside, or with the corresponding 3,4-di-*O*-acetyl compound.[92] The isomeric disaccharide linked through position 3 has likewise been synthesised from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside.[93] Derivatives of 6-*O*-(2-amino-2-deoxy-β-D-galactopyranosyl)-D-galactose which have the amino-functions in non-reducing units were synthesised by use of compound (13) and 1,2,3,4-tetra-*O*-acetyl-D-galactose and the 1,2:3,4-di-*O*-isopropylidene compound. Attempts to remove the dichloroacetyl group from the products with barium hydroxide surprisingly caused complete rupture of the glycosidic bonds. By hydrogenation the blocking group was converted into the acetyl group.[94]

For the synthesis of a diamino-dideoxy-disaccharide having amino groups in each sugar moiety, the oxazoline method was adopted (Scheme 5).[95] Various steroidal 2-acetamido-2-deoxy-D-glucopyranosides were



Scheme 5

[92] E. S. Rachaman and R. W. Jeanloz, *Carbohydrate Res.*, 1969, **10**, 435.
[93] E. S. Rachaman and R. W. Jeanloz, *Carbohydrate Res.*, 1969, **10**, 429.
[94] A. J. Acher and D. Shapiro, *J. Org. Chem.*, 1969, **34**, 2652.
[95] S. E. Zurabyan, T. P. Volosyuk, and A. J. Khorlin, *Carbohydrate Res.*, 1969, **9**, 215.

prepared using the corresponding glucosyl chloride derivatives. β-Products were thus obtained, and the anomers were prepared after titanium-tetrachloride-catalysed anomerisations. For comparison, the analogous cyclohexyl glycosides were synthesised.[96] An improved synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside has also been reported.[97]

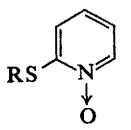
A review has appeared on the general chemistry and biochemistry of phenolic glycosides.[98] An interesting modification of the Koenigs–Knorr reaction has been applied in this area. Trimethylsilylglycosyl halides, prepared from the reaction of trimethylsilyl ethers of ethyl 1-thioglycosides with bromine, have been used to obtain 2,4-dinitrophenyl glycosides.[99] *p*-Nitrophenyl glycosides of 3-deoxy-D-*arabino*-, -D-*ribo*-, and -D-*xylo*-hexose have been prepared more conventionally for use in immunochemical studies,[100] and for similar reasons some deoxy- and dideoxy-derivatives of *p*-aminophenyl α-D-galactopyranoside have been synthesised. These were the 2-, 4-, and 6-deoxy-, and the 2,6- and 4,6-dideoxy-compounds.[101] β-D-Xylopyranosides of *p*-hydroxyacetophenone and of 4-(tri-*O*-acetyl-β-D-xylopyranosyloxy)-3-hydroxyacetophenone have also been described,[102] as has *p*-acetamidophenyl β-D-glucopyranosiduronic acid.[103]

Modified Koenigs–Knorr reaction conditions have been used for the synthesis of *p*-bromophenyl β-glycosides of cellobiose, lactose, and maltose,[104] and other aryl disaccharide glycosides were prepared by condensing tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside. Approximately equal proportions of *p*-nitrophenyl β-sophoroside and β-laminaribioside were obtained.[105] The synthesis of 4-hydroxyphenyl β-D-gentiobioside has also been described.[106]

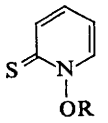
Further work has been reported on the O → N glycosyl migration reaction which takes place with some heterocyclic glycosides; in particular, the isomerisation of 2-hydroxypyridine β-D-glucopyranoside in the presence of mercuric salts has been studied.[107] Related 2-hydroxypyridine glycosides of 2-deoxy-D-*erythro*-pentose have been reported together with their *N*-bonded isomers (p. 86). Glycosylation with glycosyl halides of pyridine-2-thiol

[96] G. Sauer, M. Matsui, R. Bloch, J. S. Liang, and D. K. Fukushima, *J. Org. Chem.*, 1969, **34**, 3525.
[97] R. Begbie, *Carbohydrate Res.*, 1969, **10**, 311.
[98] S. M. Hopkinson, *Quart. Rev.*, 1969, **23**, 98.
[99] W. Hengstenberg and K. Wallenfels, *Carbohydrate Res.*, 1969, **11**, 85.
[100] G. Fahrenheim and O. Westphal, *Annalen*, 1968, **720**, 177.
[101] G. Siewert and O. Westphal, *Annalen*, 1968, **720**, 188.
[102] E. Lada and W. Wieniawski, *Acta Polon. Pharm.*, 1969, **26**, 123.
[103] L. Weintraub, S. R. Oles, A. Wilson, and L. Wilson, *J. Chem. Soc.* (*C*), 1969, 1562.
[104] I. C. M. Dea, *Carbohydrate Res.*, 1969, **11**, 363.
[105] R. N. Iyer and I. J. Goldstein, *Carbohydrate Res.*, 1969, **11**, 241.
[106] H. Frenzel and G. Wagner, *Die Pharmazie*, 1969, **24**, 235.
[107] T. Kishikawa, Y. Oikawa, and S. Takitani, *Chem. and Pharm. Bull.* (*Japan*), 1969, **17**, 699.

1-oxide gave a series of *O*- and *S*-linked glycosides, *e.g.* (14) and (15), which have antifungal and antibacterial properties.[108] 4-Hydroxy-quinazolines have been glycosidically linked with different D-glucose derivatives.[109]



(14)



(15)

R = β-D-glucopyranosyl

Two methods have been used to obtain 3-*O*-D-glucopyranosyl-D-arabinose, both of which started with other disaccharides. Ruff degradation of calcium maltobionate gave the above compound as the only product (which was isolated crystalline in both anomeric forms),[110] and application of the Fischer–Sowden procedure to 2-*O*-α-D-glucopyranosyl-D-erythrose gave the required disaccharide together with the epimeric glucosyl-ribose (each in 25% yield).[111]

A survey has been published of the chemistry of the cyanogenic glycosides, *i.e.* compounds which are glycosides of aldehyde or ketone cyanohydrins. Their enzymic degradation and their biosynthesis have also been described.[112]

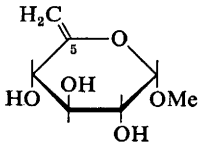
Several enzymically catalysed glycosyl transfer reactions have been examined. Use of the two anomers of D-glucopyranose and short reaction times have led to the conclusion that specific anomeric modifications are required for the enzymic dimerisations which occur in concentrated solutions in the presence of glycosidases. A crystalline glucoamylase catalysed the rapid synthesis of maltose from β-D-glucopyranose; the reaction with the α-anomer was appreciably slower. Similarly, a crystalline amylase from sweet potato catalysed the synthesis of maltotetraose from β-maltose, whereas another obtained from pig pancreas utilised α-maltose. Equilibrium constants of the reactions involved were reported.[113] The enzymic transfer of a glucose residue from maltose to 2-amino-2-deoxy-D-glucose has been used to obtain 2-amino-2-deoxy-6-*O*-(α-D-glucopyranosyl)-D-glucose,[114] and enzymes from *L. mesenteroides* have been found capable of transferring α-D-glucopyranosyl residues from sucrose to the anomeric hydroxy-groups of various free sugars. α-D-Glucopyranosyl β-D-galactofuranoside and β-D-mannopyranoside were obtained in this way.[115] The products of reaction of the β-D-fructofuranosidase from

[108] C. W. Pluijgers, J. Berg, and G. D. Thorn, *Rec. Trav. chim.*, 1969, **88**, 241.
[109] G. Wagner and F. Süss, *Die Pharmazie*, 1969, **24**, 35.
[110] H. S. Isbell, H. L. Frush, and J. D. Moyer, *J. Res. Nat. Bur. Stand. Sect. A.*, 1968, **72**, 769.
[111] I. Furda and M. B. Perry, *Canad. J. Chem.*, 1969, **47**, 2891.
[112] E. E. Conn, *J. Agric. Food Chem.*, 1969, **17**, 519.
[113] E. J. Hehre, G. Okada, and D. S. Genghof, *Arch. Biochem. Biophys.*, 1969, **135**, 75.
[114] A. L. Tarentino and F. Macey, *Arch. Biochem. Biophys.*, 1969, **130**, 80.
[115] Y. Iriki and E. J. Hehre, *Arch. Biochem. Biophys.*, 1969, **134**, 130.

yeast on a 10% solution of sucrose have been analysed and the results confirm the hypothesis that the enzyme is a transferase which first forms an enzyme–fructose complex, liberating D-glucose, and then transfers the fructose either to water or to the substrate sucrose.[116]

Hydrolysis and Anomerisation.—A review has been published on the factors affecting the acid hydrolysis of glycosides.[117]

Treatment of (16) with methanol in the presence of an acid catalyst gave (17) and (18), which were found to be interconvertible without anomerisation at C-1. This indicated that the ion (19) was involved in the isomerisation rather than an acyclic species, *e.g.* (20), and illustrated the influence of



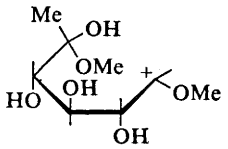
(16)

(17)

+

(18)

(19)



(20)

the C-6 group on the stabilisation of the carbonium ion. The anomerisation of glycosides was discussed in detail.[118] Consistent with this and with other information collected in recent years, methyl α- and β-D-xylopyranoside and -L-arabinopyranoside were found to anomerise by way of cyclic carbonium ions. The experiments were carried out with ^{14}C-labelled methanol.[119]

Acid-catalysed hydrolysis of sucrose in $H_2{}^{18}O$ proceeded as expected in view of the high lability of furanosyl bonds, and the fructofuranosyl–oxygen bond cleaved three times faster than the glucopyranosyl linkage.[120] The hydrolysis was also studied at limited water concentrations as a model for the reaction in foodstuffs. Saturated aqueous sucrose solutions containing acids and inert solids gave the same values of rate constants and energy of activation as those predicted from studies in dilute solutions. Rates in freeze-dried systems humidified to low moisture contents indicated that kinetic expressions describing the rate of hydrolysis must include a term for the dissolution of the solid sucrose into the surface water.[121]

[116] B. Andersen, N. Thiesen, and P. E. Broe, *Acta Chem. Scand.*, 1969, **23**, 2367.
[117] J. Szejtli, *Kem. Kozlem.*, 1969, **31**, 83 (*Chem. Abs.*, 1969, **71**, 13,286*p*).
[118] J. Lehmann, E. Pfeiffer, and H. Reinshagen, *Chem. Ber.*, 1969, **102**, 2745.
[119] M. Szymcsyk and A. Temeriusz, *Roczniki Chem.*, 1969, **43**, 1227.
[120] F. Eisenberg jun., *Carbohydrate Res.*, 1969, **11**, 521.
[121] T. Schoebel, S. R. Tannenbaum, and T. P. Labuza, *J. Food Sci.*, 1969, **34**, 324.

Rate coefficients and kinetic parameters have been determined for the acid hydrolysis of twenty-eight substituted phenyl β-D-xylopyranosides. Application of the Hammett–Zucker, the Bunnett, and entropy criteria indicated the operation of *A*-1 mechanisms for the majority of compounds. Others, however, may hydrolyse by a different process.[122] Full details of work, previously reported in preliminary form, on intramolecular catalysis in the hydrolysis of glycosides have been published; 2-carboxyphenyl α- and β-D-glucopyranoside were studied. Experiments were also carried out on some formaldehyde acetals, which suggested that they, and the glucosides, reacted by a mechanism involving intramolecular general acid catalysis.[123]

A review lecture has been published on the application of α-halogeno-ethers in carbohydrate chemistry. The cleavage of glycosidic bonds with these reagents was considered in particular.[124] More specifically, dihalogeno-methyl methyl ethers have been investigated as selective reagents for cleaving oligosaccharides from flavonoid glycosides. Rutinose (6-*O*-α-L-rhamnopyranosyl-D-glucose) hepta-acetate reacts very rapidly with dibromomethyl methyl ether to give the acetylated disaccharide α-bromide, whereas neohesperidose (2-*O*-α-L-rhamnopyranosyl-D-glucose) hepta-acetate reacted much less readily.[125]

The actions of several glycosidases have been examined in detail. N.m.r. spectroscopy has proved to be a powerful method for determining the anomeric configurations of sugars released during enzymic hydrolysis of some D-glucopyranosides and glucans. D-Glucosidases and polysaccharidases of the *endo*-type yielded products in which there was retention of the anomeric configuration of the substrates, whereas polysaccharidases of the *exo*-type caused inversions of configuration. It was proposed that these stereochemical differences may have originated in differing affinities of the enzymes for various regions of the substrates. For example, *exo*-enzymes may associate strongly with sugar and aglycone, whereas *endo*-enzymes may have strong affinity only for the sugar.[126] In a related, but more specific, study Japanese workers have analysed for phenol released during the enzymolysis of the aromatic glycosidic bond of phenyl α-maltoside by the α-amylase of *B. subtilis*, have followed the optical rotational changes occurring during the process, and have shown that the reaction occurred with retention of configuration, *i.e.* α-maltose was released exclusively. The enzyme responsible was studied in considerable detail.[127] α-Amylase activity on several partially methylated phenyl α-maltosides

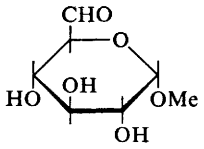
[122] F. Van Wijnedaele and C. K. De Bruyne, *Carbohydrate Res.*, 1969, **9**, 277.
[123] B. Capon, M. C. Smith, E. Anderson, R. H. Dahm, and G. H. Sankey, *J. Chem. Soc.* (*B*), 1969, 1038.
[124] F. Istvan, R. Bognar, I. F. Szabo, and M. Menyhart, *Kem. Kozlem*, 1968, **30**, 297 (*Chem. Abs.*, 1969, **70**, 78,259*f*).
[125] B. H. Koeppen, *Carbohydrate Res.*, 1969, **10**, 105.
[126] D. E. Eveleigh and A. S. Perlin, *Carbohydrate Res.*, 1969, **10**, 87.
[127] K. Hiromi, T. Shibaoka, H. Fukube, and S. Ono, *J. Biochem.* (*Japan*), 1969, **66**, 63, 183.

has also been examined, and it was concluded that all the hydroxy-groups on the maltose reducing moiety and that at C-2 of the non-reducing moiety must be free before hydrolysis occurs.[128] A kinetic study of the hydrolysis of 5-bromo-4-chloroindol-3-yl β-D-glucopyranoside by almond emulsin has demonstrated the potential utility of the 'indigogenic principle' in the evaluation of the action of other hydrolytic enzymes.[129] A β-xylosidase obtained from the liver of *Charonia lampas*, a marine gastropod, has been isolated and freed from all other glycosidase activity except towards β-glucosides. It hydrolysed a xylosyl protein (*O*-β-D-xylopyranosyl-L-serine) and xylan. Both the xylosidase and glucosidase activities were assigned to the same enzyme.[130]

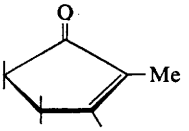
Other Reactions and Features of Glycosides.—Primary reactions occurring during the radiolysis of cellobiose, maltose, gentiobiose, and lactose have been studied in solution by chemical methods and in frozen solutions by e.s.r. spectroscopy. Reactions occurred initially with hydroxyl radicals, hydrogen atoms, and hydrated electrons, and disproportionations and rearrangements which followed were discussed; deoxy- and deoxyketo-products were formed.[131] The aldehyde (21) is a new product detected in the radiolysis products of methyl α-D-glucopyranoside.[132] On irradiation with u.v. light in the presence of hydrogen peroxide and iron(II) sulphate, *p*-nitrophenyl β-D-glucopyranosiduronic acid underwent hydrolysis.[133] The same glycoside was stable on irradiation in acid and neutral media, but was quantitatively cleaved to give *p*-nitrophenol in alkaline media. Recovery of the carbohydrate was very inefficient.[134]

Sucrose has been caramelised and the products extracted with 84% ethanol to give a series of glucobioses, other disaccharides, and anhydro-derivatives.[135] The volatile products formed on pyrolysis of the sugar resemble those obtained on aqueous tin(II) chloride degradation of glucose; three previously unrecognised products (22)—(24) were characterised.[136] Compounds (25) and (26) were found, together with dihydroxyacetone, acetic, propionic, and lactic acids amongst the ether-soluble products after treatment [137] of sucrose at pH 11·5 and at 100 °C.

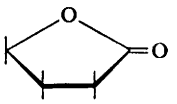
[128] M. Isemura, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1969, **66**, 77.
[129] J. P. Horwitz, C. V. Easwaran, and L. S. Kowalczyic, *Carbohydrate Res.*, 1969, **9**, 305.
[130] M. Fukuda, T. Muramatsu, and F. Egami, *J. Biochem.* (*Japan*), 1969, **65**, 191; **66**, 157.
[131] N. K. Kochetkov, L. I. Kudriashov, S. M. Yarovaya, M. T. Nadgymiddinova, V. A. Sharpatii, and N. M. Emanuel, *Khim. vysok. Energii*, 1968, **2**, 556, 286.
[132] L. I. Kudriashov, M. A. Chlenov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 189.
[133] Y. Yamane, M. Miyazaki, K. Sakai, and C. Ajiki, *J. Pharm. Soc. Japan*, 1969, **89**, 863.
[134] Y. Yamane, M. Miyazaki, K. Sakai, C. Ajiki, L. Z. Chang, and K. Yoshimoto, *Chem. and Pharm. Bull.* (*Japan*), 1969, **17**, 1183.
[135] S. Kitaoka and K. Suzuki, *Shimane Noka Daigaku Kenkyu Hokoku*, 1967, **15**, 19 (*Chem. Abs.* 1969, **70**, 11,918*b*).
[136] R. R. Johnson, E. D. Alford, and G. W. Kinzer, *J. Agric. Food Chem.*, 1969, **17**, 22.
[137] P. E. Shaw, J. H. Tatum, and R. E. Berry, *J. Agric. Food Chem.*, 1969, **17**, 907.



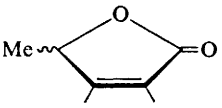
(21)



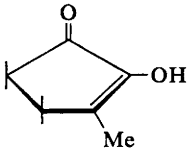
(22)



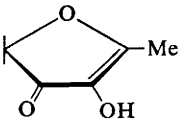
(23)



(24)



(25)



(26)

Certain methyl glycofuranosides have been found to be inhibitors of bacterial growth.[138] A further finding of physiological interest has been that flavanone glycosides of 2-*O*-α-L-rhamnosyl-β-D-glucose, found in citrus fruits, were bitter in taste, whereas isomeric glycosides of 6-*O*-α-L-rhamnosyl-β-D-glucose were tasteless. Other aspects of the taste–structure correlation of these compounds were discussed.[139]

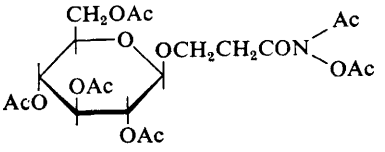
The o.r.d., c.d., and u.v. spectra of phenyl and *o*- and *p*-nitrophenyl α- and β-D-hexopyranosides have been studied in order to explain why *o*-nitrophenyl glycosides exhibit anomalous optical rotational behaviour. It was shown that the *o*-nitro group is twisted from the plane of the benzene ring, and so itself becomes an optically active chromophore exhibiting a Cotton effect at 340 nm, thus affecting the $[\alpha]_D$ values of these glycosides.[140] The o.r.d. of some other phenyl glycosides is mentioned on p. 182.

Natural Products.—A vast amount of new information continues to be reported on naturally occurring glycosides. In particular, Reichstein's group has produced highly detailed evidence on the structures of new compounds. Their work appears in *Helvetica Chimica Acta*, and each issue of *Chemical Abstracts* contains additional reports on this subject. Here, only a few new glycosides of interest from the carbohydrate point of view are noted.

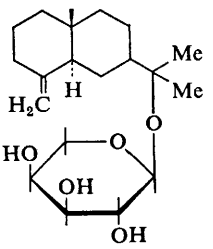
A new disaccharide, maniocose, isolated from manioc flour, has been shown to be 5-*O*-(α-D-glucopyranosyl)-D-glucose,[141] and the compound has been synthesised from 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose by treatment with Brigl's anhydride or 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride.[142]

[138] D. H. Murray, *J. Pharm. Sci.*, 1969, **58**, 775.
[139] R. M. Horowitz and B. Gentili, *J. Agric. Food Chem.*, 1969, **17**, 696.
[140] Y. Tsuzuki, M. Koyama, K. Aoki, T. Kato, and K. Tanabe, *Bull. Chem. Soc. Japan*, 1969, **42**, 1052.
[141] J. Jadot and G. Maghuin-Rogister, *Bull. Soc. chim. belges*, 1968, **77**, 569.
[142] G. Maghuin-Rogister, *Bull. Soc. chim. belges*, 1968, **77**, 575.

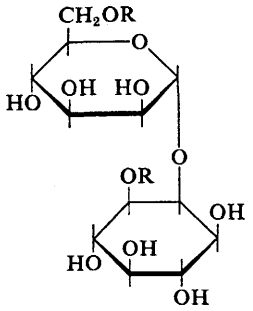
Miserotoxin, a toxic substance found in timber milkvetch, which can poison livestock, has been identified as 3-nitropropyl β-D-glucopyranoside. Treatment with acetic anhydride and sodium acetate surprisingly gave (27).[143] (+)-β-Eudesmol α-L-arabinopyranoside (28) has been obtained from *Machaeranthera tanacetifolia*,[144] and a diacyl myoinositol monomannoside (29) was isolated from *Propionibacterium shermanii*.[144a]



(27)

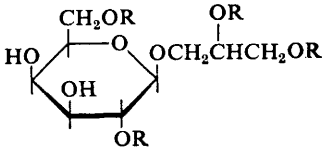


(28)



(29)

R = fatty acid radical



(30)

R = fatty acid radical

Several glycerol derivatives have been identified. Compound (30) has been obtained from spinach leaves,[145] and 1-[*O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl]glycerol has been isolated from the glycolipids of two *Streptococci*.[146] Amongst the products formed when *Streptococcus lactis* was grown on D-galactose as its carbon source were 4-*O*-β-D-glucopyranosyl-α,α-trehalose and homologues extending from the *O*-α-maltosyl analogue.[147]

S-Glycosides

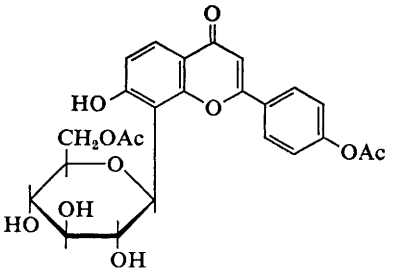
The chemistry of the naturally occurring thioglycosides and their biological effects on ingestion have been reviewed.[148] The Koenigs–Knorr reaction

[143] F. R. Stermitz, F. A. Norris, and M. C. Williams, *J. Amer. Chem. Soc.*, 1969, **91**, 4599.
[144] H. Yoshioka, T. J. Mabry, and A. Higo, *J. Org. Chem.*, 1969, **34**, 3697.
[144a] C. Prattey and C. E. Ballou, *J. Biol. Chem.*, 1968, **243**, 6196.
[145] E. Heinz and A. P. Tulloch, *Z. physiol. Chem.*, 1969, **350**, 493.
[146] W. Fisher and W. Seyferth, *Z. physiol. Chem.*, 1968, **349**, 1662.
[147] W. Fisher and K. Winkler, *Z. physiol. Chem.*, 1969, **350**, 1137.
[148] C. H. VanEtten, M. E. Daxenbichler, and I. A. Wolff, *J. Agric. Food Chem.*, 1969, **17**, 483.

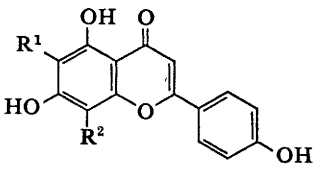
has been used to synthesise phenyl and *o*-nitrophenyl 1-thio-β-D-galactopyranoside and *o*-nitrophenyl 1-thio-β-D-fucopyranoside,[149] and a series of disaccharide phenyl thioglycosides have been prepared from cellobiose, maltose, and lactose.[150] Condensation of acylated glycosyl halides with pyridine-2-thiol 1-oxide gave thioglycosides in addition to *O*-glycosides,[108] and glycosides of 4-mercaptoquinazolines have been prepared in similar fashion.[109]

C-Glycosides

New *C*-glycosides continue to be found in plant extracts. The roots of *P. tuberosa* yielded a compound characterised as 4′,6″-di-*O*-acetylpuerarin (31),[151] and compound (32) has been isolated from lemon rind and has also been synthesised by condensation between tetra-*O*-acetyl-α-D-glucopyranosyl bromide and vitexin (33).[152] Two interconvertible 6,8-di-*C*-glucosyl-



(31)

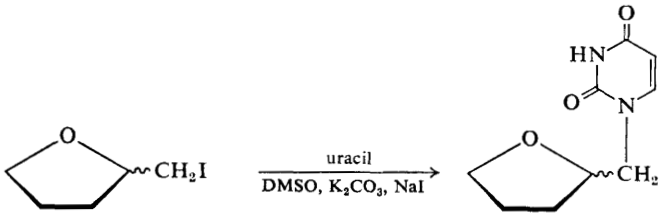


(32) $R^1 = R^2 =$ β-D-glucopyranosyl
(33) $R^1 = H$, $R^2 =$ β-D-glucopyranosyl

5,7,4′-trihydroxyflavones containing two different sugar components have been obtained from liverwort.[153] A series of ^{14}C-labelled flavones have been introduced into the growth media of various plants, and *O*-glycosylated compounds were produced. No *C*-glycosyl derivatives were formed, which suggests that *C*-glycosides are not synthesised by glycosylation of the flavones.[154]

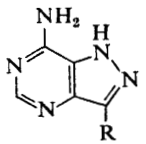
A series of derivatives of uracil, thymine, and cytosine have been prepared as exemplified in Scheme 6; they can be considered to be nucleoside analogues with a carbon atom separating the furanose and base rings.[155] Other *C*-linked nucleoside analogues are the antibiotics formycin (34), formycin B (35), and showdomycin (36), which have now been examined by mass spectrometry. All show base peaks at m/e = (heterocyclic base + 30),

[149] N. Janaki, J. R. Patil, and J. L. Bose, *Indian J. Chem.*, 1969, **7**, 227.
[150] G. Wagner and R. Metzner, *Die Pharmazie*, 1969, **24**, 245.
[151] S. P. Bhutani, S. S. Chibber, and T. R. Seshadri, *Indian J. Chem.*, 1969, **7**, 210.
[152] J. Chopin, B. Roux, M. L. Bouillant, A. Durix, A. d'Arcy, T. Mabry, and H. Yoshioka, *Compt. rend.*, 1969, **268**, *C*, 980.
[153] K. R. Markham, L. J. Porter, and B. G. Brehm, *Phytochemistry*, 1969, **8**, 2193.
[154] J. W. Wallace, T. J. Mabry, and R. E. Alston, *Phytochemistry*, 1969, **8**, 93.
[155] J. Defaye, M. Naumberg, and T. Reyners, *J. Heterocyclic Chem.*, 1969, **6**, 229.

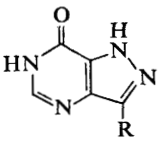


Scheme 6

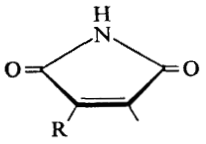
corresponding to the heterocycles substituted with protonated formyl groups. By contrast, *N*-linked nucleosides usually give base peaks at *m/e* = (base+1) or (base+2). The method, therefore, is of appreciable value in structural studies of compounds of this series.[155a]



(34)



(35)



(36)

R = β-D-ribofuranosyl

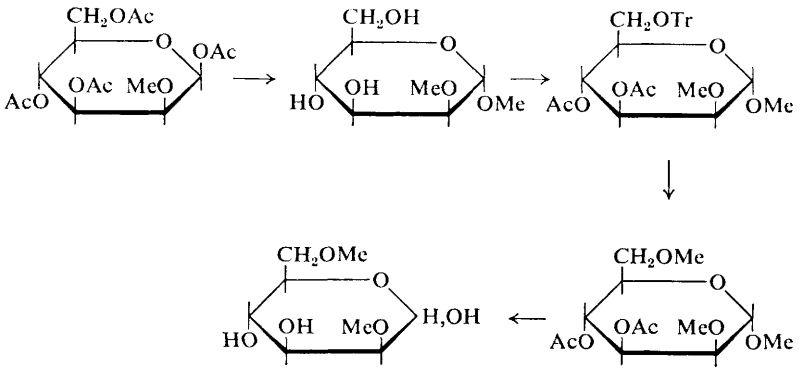
An unsaturated C–C-linked disaccharide derivative has been found amongst the products of treatment of tri-*O*-acetyl-D-glucal with boron trifluoride etherate (see p. 111).

[155a] L. B. Townsend and R. K. Robbins, *J. Heterocyclic Chem.*, 1969, **6**, 459.

4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—Amongst the methyl ethers prepared this year were several D-mannose derivatives. The 5-*O*-methyl ether was synthesised by way of methyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-mannofuranoside.[74] 2,6-Di-*O*-methyl-D-mannose was prepared as outlined in Scheme 7; apart



Scheme 7

from the methylation at the primary site which was carried out with diazomethane, with boron trifluoride etherate as catalyst, all the reactions were conducted under standard conditions.[156] The 2,4-disubstituted isomer was obtained by successive methylation, desulphonylation, detritylation, and hydrolysis of methyl 3-*O*-toluene-*p*-sulphonyl-6-*O*-trityl-α-D-mannopyranoside.[156a] The syntheses of the 2,3- and 3,4-di-*O*-methyl-mannoses have also been reported.[157] In a paper of wider scope, the preparations of a selection of di- and tri-*O*-methyl-mannoses were described. Extensive use was made of the conversion of benzylidene acetals to monobenzyl ethers, (see later), which were methylated prior to removal of

[156] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1969, **47**, 31.
[156a] V. L. N. Murty and I. R. Siddiqui, *Carbohydrate Res.*, 1969, **10**, 477.
[157] V. L. N. Murty and I. R. Siddiqui, *Carbohydrate Res.*, 1969, **11**, 273.

the benzyl protecting groups. The products were used for characterising methyl ethers obtained from methylated mannans. Initially these ethers were fractionated into di-, tri-, and tetra-*O*-methyl fractions before g.l.c. analysis.[158] 3,5,6-Tri-*O*-methyl-D-mannose was independently prepared from methyl 3,5-di-*O*-methyl-2-*O*-toluene-*p*-sulphonyl-6-*O*-trityl-α-D-manno-furanoside.[159]

Mixtures of methyl ethers of methyl α-D-mannopyranoside have been synthesised in an assessment of the relative activities of the hydroxy-groups in the glycoside. With methyl sulphate and sodium hydroxide the relative substitution order was $6>2>3>4$, whereas with methyl iodide and silver oxide in DMF it changed to $2>3>4\geqslant 6$. Methyl iodide and methylsulphinyl carbanion in DMSO gave $2>6>4\geqslant 3$.[160]

In the D-galactose series an improved synthesis of the 3-*O*-methyl ether from methyl 4,6-*O*-benzylidene-3-*O*-(ethoxycarbonyl)-β-D-galactopyranoside has been described. The 2-position was protected by conversion into the tetrahydropyranyl ether, and the ester group was removed by treatment with potassium carbonate prior to methylation.[161] 4-*O*-Methyl-D-galactose and the 4,6-di-*O*-methyl ether have been prepared by using diazomethane and boron trifluoride etherate on partially benzoylated galactose derivatives.[162]

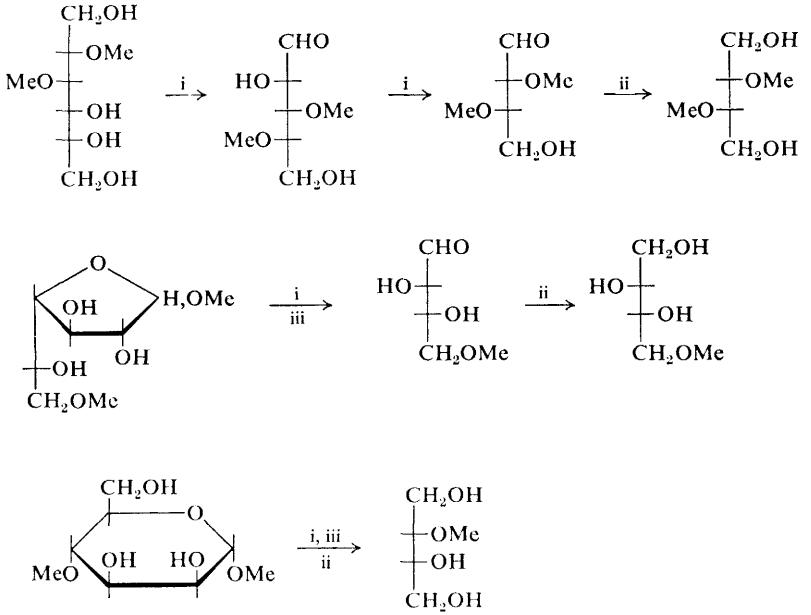
N.m.r. assignments for the ten signals derived from the methyl groups in methyl 2,3,4,6-tetra-*O*-methyl-α- and -β-D-glucopyranoside have been given, based on experiments in which various groups were selectively deuteriated. The information was then used to analyse the products of hydrolysis of tri-*O*-methyl-cellulose and to assess the pattern of methylation in partially methylated methyl β-D-glucopyranoside.[163]

6-Deoxy-2,4-di-*O*-methyl-D-allopyranose has been synthesised by two routes; the 2,3- and 3,4-disubstituted isomers were also obtained in the course of these studies. 2,4-Phenylboronate esters and 3,4-*O*-isopropylidene acetals were used as protecting groups.[164]

Some methylated tetroses, tetritols, and pentoses have been synthesised as shown in Scheme 8, for use as reference compounds in the characterisation of products from periodate oxidation of partially methylated sugars produced in the course of polysaccharide structural work.[165]

Methyl ethers of 2-amino-2-deoxy-D-glucose, obtained by the hydrolysis of methylated polymers which contain the sugar, were examined by g.l.c. of the fully acetylated 2-acetamido-2-deoxy-D-glucitol derivatives. A further means of characterising such sugar ethers involved treating them

[158] S. S. Bhattacharjee and P. A. J. Gorin, *Canad. J. Chem.*, 1969, **47**, 1207.
[159] I. R. Siddiqui, *Carbohydrate Res.*, 1969, **9**, 344.
[160] N. Handa and R. Montgomery, *Carbohydrate Res.*, 1969, **11**, 467.
[161] E. G. Gros and I. O. Mastronardi, *Carbohydrate Res.*, 1969, **10**, 325.
[162] E. G. Gros and I. O. Mastronardi, *Carbohydrate Res.*, 1969, **10**, 318.
[163] D. Gagnaire and L. Odier, *Carbohydrate Res.*, 1969, **11**, 33.
[164] J. S. Brimacombe, F. Hunedy, and A. Husain, *Carbohydrate Res.*, 1969, **10**, 141.
[165] G. G. S. Dutton, K. B. Gibney, and P. E. Reid, *Canad. J. Chem.*, 1969, **47**, 2494.



Reagents: i, $NaIO_4$; ii, $NaBH_4$; iii, H^+

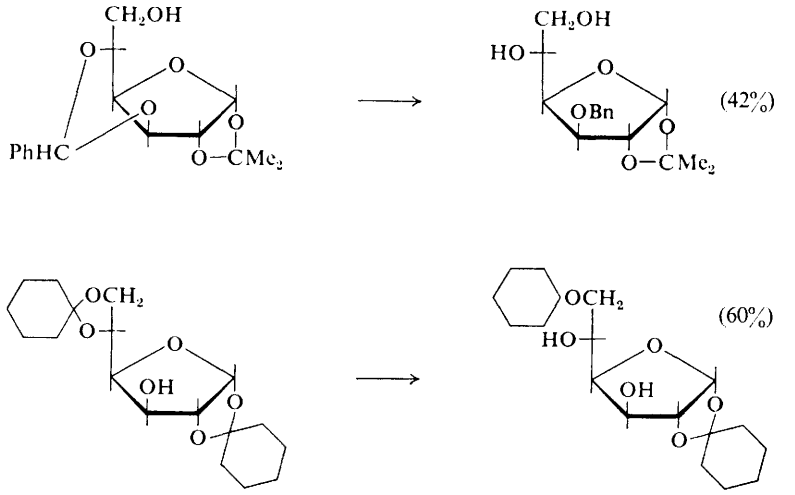
Scheme 8

with ninhydrin and examining the resultant D-arabinose methyl ethers by paper chromatographic methods.[166] The 3-, 4-, and 6-monoethers, the 3,4-, 3,6-, and 4,6-diethers, and the 3,4,6-triether, of 2-deoxy-2-(2,4-dinitro-anilino)-D-glucose and its ethyl α-glucoside have been prepared for use in the analysis of amino-sugar-containing polysaccharides.[167]

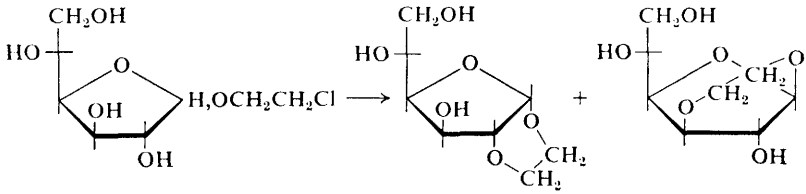
Substituted Alkyl Ethers.—The reaction of carbohydrate acetal derivatives with lithium aluminium hydride and aluminium trichloride to give the corresponding ethers has been examined and found to provide a means for obtaining selectively substituted derivatives which are not otherwise readily available.[168] The use of this reaction has already been indicated in the previous section; specific examples are given in Scheme 9.

Further attention has been given to the cyclic ethers formally produced on condensing ethylene glycol with the C-1,C-2 diol system of D-glucose. As was noted in Chapter 3, acid treatment of 2-*O*-(2-hydroxyethyl)-D-glucose gave a mixture of furanoid and pyranoid derivatives.[73] Such compounds were also prepared from β-chloroethyl glycosides by treatment with base as shown in Scheme 10.[169]

[166] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1969, **47**, 4091.
[167] P. F. Lloyd, B. Evans, and R. J. Fielder, *Carbohydrate Res.*, 1969, **9**, 471.
[168] S. S. Bhattacharjee and P. A. J. Gorin, *Canad. J. Chem.*, 1969, **47**, 1195.
[169] H. C. Srivastava, K. V. Ramalingam, and A. S. Chaudhari, *Tetrahedron Letters*, 1969, 2643.



Scheme 9



Scheme 10

Further studies of the vinylation of methyl α-D-glucopyranoside with acetylene and potassium hydroxide in aqueous dioxan at high temperatures and pressures have resulted in the isolation of methyl 3-*O*-vinyl-α-D-glucopyranoside.[170] Perallyl ethers of several pyranosides have been prepared using the allyl bromide in alkaline solution, and from these, propyl derivatives were obtained by hydrogenation.[171] High molecular weight products have been prepared by polymerisation of 6-*O*-allyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactose.[172]

5′-Carboxymethyl derivatives of adenosine, guanosine, cytidine, and uridine have been prepared by oxidation of the corresponding β-hydroxyethyl ethers and are considered to be nucleotide analogues.[173]

[170] J. T. Marrel, J. W. Berry, R. O. Kuehl, and A. J. Deutszchman jun., *Carbohydrate Res.*, 1969, **9**, 295.
[171] B. I. Mikhant'ev, V. L. Lapenko, and V. E. Sopina, *Izvest. V.U.Z., Khim. i khim. Tekhnol.* 1969, **12**, 603 (*Chem. Abs.*, 1969, **71**, 70,884*f*).
[172] W. A. P. Black, J. A. Colquhoun, and E. T. Dewar, *Makromol. Chem.*, 1969, **122**, 244.
[173] J. P. Coat and S. David, *Compt. rend.*, 1969, **268**, *C*, 1160.

Reaction of sodium sucrate with long-chain alkyl bromides in DMSO gave surface-active ethers (octyl, decyl, *etc.*).[174]

Silyl Ethers.—The use of DMF or DMSO as solvents for trimethylsilylation of sugars with hexamethyldisilazane or trimethylsilyl chloride gives an upper phase of hexamethyldisiloxane into which the TMS ethers of monosaccharides partition. In this way they can be concentrated and removed from the primary reaction solvents. Other aspects of the reactions were examined in detail.[175] The mass spectra of a series of trimethylsilyl derivatives of free sugars and glycosides have been investigated and the fragmentation patterns elucidated with the aid of deuterium labelling and exact mass studies. In conjunction with g.l.c., mass spectrometry was then utilised to examine the products of trimethylsilylation of free sugar and glycoside mixtures. Furanosyl derivatives were encountered in all cases. From D-glucose, the furanosyl ethers were present to the extent of 5%, indicating that the procedure did not reflect the equilibrium situation in water. 3-*O*-Methyl-D-glucose and 2-acetamido-2-deoxy-D-galactose were also examined. As has been shown previously, mass spectrometry offers powerful means of allocating ring sizes to trace components of mixtures.[176]

Penta-*O*-trimethylsilyl-α-D-glucopyranose and [$^2H_{45}$]penta-*O*-trimethylsilyl-α-D-glucopyranoside were completely resolvable by g.l.c., the former having the higher retention time.[177]

The trimethylsilylation of hex-2-uloses and hept-2-uloses gave initial products in which the anomeric hydroxy-groups were unsubstituted, and subsequently fully substituted ethers.[178] Coriose (D-*altro*-hept-3-ulose) behaved similarly but two final products were obtained, one the fully substituted derivative of the *keto*-modification, and the other the isomeric fully substituted α-furanose ether.[179]

The use of trimethylsilylated derivatives in synthetic work is expanding. Trimethylsilylated glycosyl halides have been used in Koenigs–Knorr reactions.[99] Condensation of trimethylsilyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside with a glycosyl halide derivative, in the presence of silver perchlorate, caused displacement of trimethylsilyl chloride to give a substituted non-reducing disaccharide.[87]

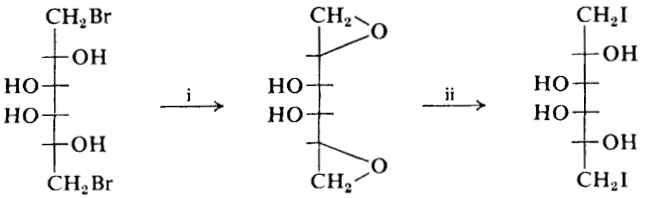
Intramolecular Ethers (Anhydro-sugars)

Epoxides.—A kinetic study has been made of the formation of epoxides from various mono- and di-toluene-*p*-sulphonates of 1,6-anhydro-β-D-glucopyranose under dilute alkaline conditions. The compounds reacted

[174] J. A. Reeder, H. B. Rayner, G. Aitken, D. Bradley, and J. Atkinson, *Ind. and Eng. Chem., Product Res. and Development*, 1968, **7**, 230 (*Chem. Abs.*, 1969, **70**, 11,924*a*).
[175] W. C. Ellis, *J. Chromatog.*, 1969, **41**, 325.
[176] D. C. De Jongh, T. Radford, J. D. Hribar, S. Hanessian, M. Bieber, G. Dawson, and C. C. Sweeley, *J. Amer. Chem. Soc.*, 1969, **91**, 1728.
[177] G. R. Waller, S. D. Sastry, and K. Kinneberg, *J. Chromatog.*, 1969, **7**, 577.
[178] T. Okuda and K. Konishi, *Chem. Comm.*, 1969, 796.
[179] T. Okuda and K. Konishi, *Chem. Comm.*, 1969, 1117.

at the following relative rates: 2-ester, 1·0; 3-ester, 180; 4-ester, 23·3; 2,4-diester, 32·8; 4-ester of the 2-deoxy-analogue, 0·6. From the 3-toluene-*p*-sulphonate a mixture of the 3,4- and 2,3-epoxides was obtained with the components present in the ratio 2·3 : 1.[180]

1,6-Dibromo-1,6-dideoxy-galactitol gave 1,2:5,6-dianhydro-galactitol on treatment with mild base, and the product was converted into the di-iodo-analogue of the starting material by nucleophilic ring opening using potassium iodide (Scheme 11). Under mild conditions 1-*O*-methanesulphonyl-D-mannitol and 3,4-di-*O*-methanesulphonyl-D-mannitol gave



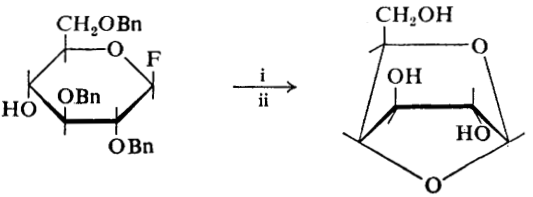
Reagents: i, pH 8; ii, KI, HCl

Scheme 11

1,2-anhydro-D-mannitol and 2,3:4,5-dianhydro-D-iditol. Under strongly alkaline conditions 1,6-di-*O*-methanesulphonyl-D-mannitol gave the enantiomeric L-ido dianhydride.[180a]

The reaction of methyl 5-*O*-acetyl-2,3-anhydro-D-ribofuranosides with magnesium bromide afforded bromo-deoxy products as described on p. 65; branched-chain products formed from epoxides are mentioned on p. 123. Exocyclic epoxides are also referred to in Chapter 15. The n.m.r. spectra of 2,3- and 3,4-anhydro-hexopyranosides are mentioned on p. 174.

Other Anhydrides.—The synthesis of 1,4-anhydro-D-glucopyranose has been accomplished by the route outlined in Scheme 12.[181, 182]



Reagents: i, NaOH; ii, H_2–Pd

Scheme 12

[180] M. Černý, J. Staněk jun., and J. Pacák, *Coll. Czech. Chem. Comm.*, 1969, **34**, 849.
[180a] M. Jarman and W. C. J. Ross, *Carbohydrate Res.*, 1969, **9**, 139.
[181] F. Micheel and U. Kreutzer, *Tetrahedron Letters*, 1969, 1459.
[182] F. Micheel and U. Kreutzer, *Annalen*, 1969, **722**, 228.

1,6-Anhydro-2-deoxy-β-D-hexopyranoses with the *arabino*, *lyxo*, and *ribo* configurations have been described. The first and third were prepared from 1,6-anhydro-4-*O*-benzyl-β-D-glucopyranose and the *lyxo* compound from the known 1,6:2,3-dianhydro-β-D-talopyranose. Also described was the synthesis of 1,6:2,3- and 1,6:3,4-dianhydro-β-D-allopyranose from 1,6-anhydro-4-*O*-benzyl-2,3-di-*O*-toluene-*p*-sulphonyl-β-D-glucopyranose.[183] Closely related results were reported in the section on epoxides above. The possible transition of 1,6-anhydro-β-D-glucopyranose from a chair to a boat conformation in solution and in the crystal has been examined, mainly by i.r. methods.[184] Other conformational aspects of such compounds are reported in Chapter 23.

The suggestion that 1,2-anhydro-derivatives are necessary intermediates in the thermal depolymerisation of cellulose to 1,6-anhydro-β-D-glucopyranose has been refuted by the finding that 1,6-anhydro-2-*O*-methyl-β-D-glucopyranose may be obtained by thermal depolymerisation of 2-*O*-methylcellulose.[185] A disaccharide synthesis which uses Brigl's anhydride has already been referred to.[142]

Polymerisation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-D-glucose, with phosphorus pentafluoride in methylene chloride at low temperatures, gave a stereoregular α-linked polymer;[186] similarly the β-D-mannopyranose anomer has been converted into a regular α-mannan.[187] In related work, 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose was fused in the presence of acidic catalysts to give 1,6-anhydro-β-D-glucopyranose as well as oligosaccharides after deacetylation.[188]

One of the two previously known crystalline modifications of 1,6-anhydro-β-D-altropyranose has been recognised as a monohydrate.[189]

Reductions of 6-substituted trehalose sulphonates, deoxy-iodo or thiocyanate derivatives, all afforded 3,6-anhydro-trehaloses.[190]

Several 5-(1,2,3,4,5-pentahydroxypentyl)-6-azauracils have been prepared and treated with hydrochloric acid. Compounds with the D-*allo*- and D-*altro*-configurations formed 2′,5′-anhydrides, whereas the D-*gluco*- and D-*galacto*-isomers did not.[191]

[183] P. A. Seib, *J. Chem. Soc.* (*C*), 1969, 2552.
[184] V. N. Nikitin, I. Yu. Levdik, and M. A. Ivanov, *Zhur. strukt. Khim.*, 1968, **9**, 1011.
[185] P. C. Wollwage and P. A. Seib, *Carbohydrate Res.*, 1969, **10**, 589.
[186] J. Zachoval and C. Schuerch, *J. Amer. Chem. Soc.*, 1969, **91**, 1165.
[187] J. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, 1969, **91**, 1161.
[188] D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohydrate Res.*, 1969, **11**, 453, 461.
[189] I. Johansson and N. K. Richtmeyer, *Carbohydrate Res.*, 1969, **10**, 322.
[190] E. R. Guilloux, F. Percheron, and J. Defaye, *Carbohydrate Res.*, 1969, **10**, 267.
[191] M. Bobek, J. Farkaš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1673.

5
Acetals

Reactions and Properties of Acetals

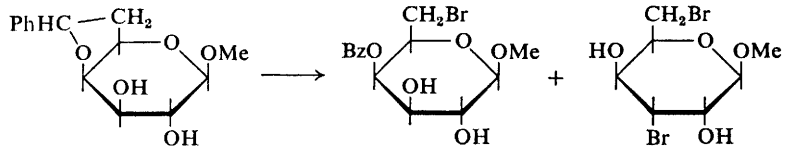
Carbohydrate derivatives, notably acetals, have been used to form complexes with reagents which add to carbonyl derivatives; the asymmetric complexes produced induce asymmetry in the products. Thus, substituted sugars such as 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside have been shown to complex strongly with Grignard reagents, so that additions of the complexes to aldehydes or ketones result in the generation of highly optically active alcohols. The full utility of this approach remains to be explored.[192] In related fashion, asymmetric reduction of substituted acetophenones with the complexes formed between 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and lithium aluminium hydride has been studied and has given alcohols with *R* : *S* ratios of *ca.* 1 : 1·1.[193]

Ring-opening reactions of acetals are becoming increasingly useful for synthetic purposes. With lithium aluminium hydride–aluminium trichloride, acetals were converted into the corresponding ethers (*e.g.* benzylidene → benzyl, cyclohexylidene → cyclohexyl) which were utilised in the synthesis of partially substituted derivatives.[158] Rates of ring-opening reactions decreased in the order isopropylidene > cyclohexylidene > benzylidene > ethylidene > methylidene, and 5,6- or 3,5-linked acetals on furanoid rings cleaved more readily than the corresponding 1,2-acetals. Acetals may be synthesised by the application of this same reaction to orthoesters (see p. 46).[168]

4,6-*O*-Benzylidene-hexopyranosides on treatment with *N*-bromosuccinimide in refluxing carbon tetrachloride generally give 4-*O*-benzoyl-6-bromo-6-deoxy-hexopyranosides in high yield. The reaction has already been applied in several instances to the synthesis of such compounds, and now its scope has been examined. Many protecting groups as well as epoxide rings remained intact under the conditions of the reaction; disaccharide acetals behaved as expected. One unexpected finding was noted with methyl 4,6-*O*-benzylidene-β-D-galactopyranoside, which gave two products in equal proportions as shown in Scheme 13. The possible

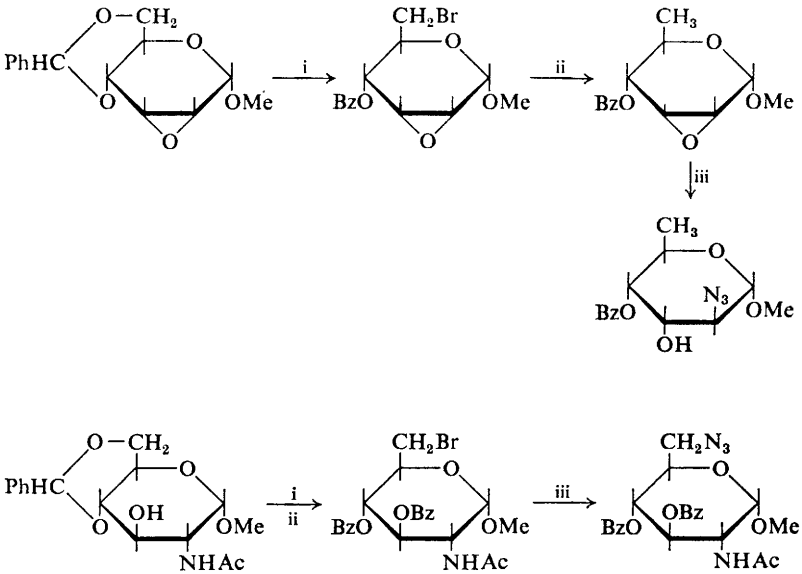
[192] T. D. Inch, G. J. Lewis, G. L. Sainsbury, and D. J. Sellers, *Tetrahedron Letters*, 1969, 3657.

[193] O. Červinka and A. Fábryová, *Z. Chem.*, 1969, **9**, 426.



Scheme 13

mechanisms of the reactions were discussed in detail.[194] Further applications have been made to give an extensive series of modified hexose derivatives, many of which are potential precursors of compounds of biological importance. Two of the many examples reported are given in Scheme 14.[195] The investigation was then extended to benzylidene acetals

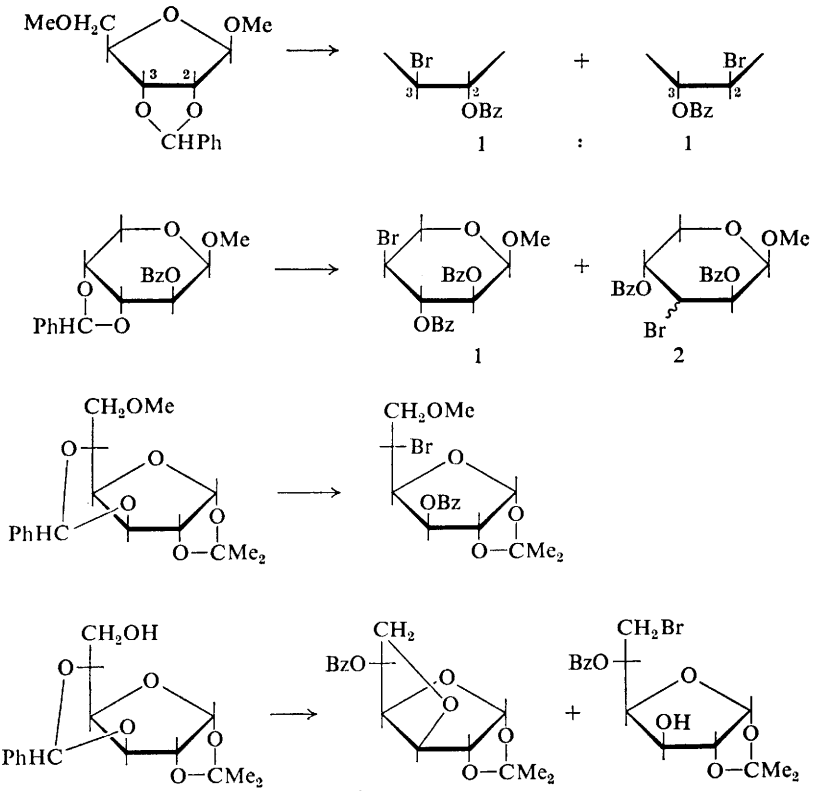


Reagents: i, NBS, CCl_4; ii, BzCl–py; iii, NaN_3, DMF

Scheme 14

formed from two secondary alcohol groups. When the oxygen atoms of the acetals were *cis*-related on sugar rings, mixtures of *trans*-bromobenzoates were obtained, whereas if one was on a ring and the other on a side-chain, the product having bromine in the side-chain was produced. Participating groups suitably situated with respect to the intermediate benzoxonium ions gave rearranged ionic intermediates, which then ring-opened by attack of bromide ion.[196] These principles are illustrated in Scheme 15.

[194] S. Hanessian and N. R. Plessas, *J. Org. Chem.*, 1969, **34**, 1035.
[195] S. Hanessian and N. R. Plessas, *J. Org. Chem.*, 1969, **34**, 1045.
[196] S. Hanessian and N. R. Plessas, *J. Org. Chem.*, 1969, **34**, 1053.



Scheme 15

N.m.r. studies of the peracetylated diethyl dithioacetals of several aldoses have revealed the preferred conformations of the compounds (p. 175).

Synthesis of Acetals

From Diols on Acyclic Carbohydrates.—A general approach has already been discussed (see p. 46).[168] Acetonation of D-ribose diethyl dithioacetal with anhydrous copper sulphate as catalyst gave mainly the 4,5- and 2,4-*O*-isopropylidene derivatives which were isolated in 65 and 12% yield by chromatography. They were characterised by chemical and n.m.r. methods.[197] The major products obtained from the acid-catalysed reactions of several alkyl vinyl ethers and D-galactose diethyldithioacetal in equimolar proportions were the 5,6-*O*-alkylidene derivatives, formed presumably by initial additions of the primary hydroxy-groups across the vinyl double bonds, followed by ring closures.[198]

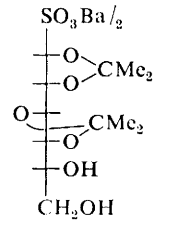
[197] D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 2889.
[198] M. L. Wolfrom and G. G. Parekh, *Carbohydrate Res.*, 1969, **11**, 547.

The bisulphite adduct of D-glucose (as its cyclohexylammonium salt) on treatment with acetone and sulphuric acid followed by neutralisation with barium carbonate gave a product believed to be (37) although the location of the acetal rings and the C-1 configuration were not determined.[199]

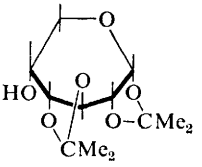
The condensation of butyraldehyde with D-mannitol has been investigated, and the 1,3:2,5:4,6-tri-, the 1,3:4,6- and 1,3:5,6-di-, and the 1,3- and 3,4-acetals have been identified amongst the products. The results suggest that the rules governing the preferential formation of acetal derivatives of polyols are followed less closely as the size of the aldehyde increases.[200]

Details of the acetylation of D-glucose phenylosotriazole with benzaldehyde are given in Chapter 9.

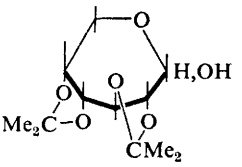
From Diols on Cyclic Carbohydrates.—(*i*) *Free Sugars.* Acetonation of D-glucose using 4% sulphuric acid as catalyst afforded, apart from the main product 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and the known syrupy 1,2:3,5-furanose isomer, two minor acetals shown to be the 1,2:3,4-di-*O*-isopropylidene-α-D-glucoseptanose (38) and the 2,3:4,5-di-*O*-isopropylidene-D-glucoseptanose isomer (39). Compound (38) was crystalline, and on partial hydrolysis gave 1,2-*O*-isopropylidene-α-D-glucoseptanose (40) which was also crystalline.[201]



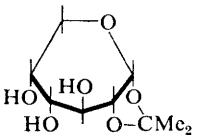
(37)



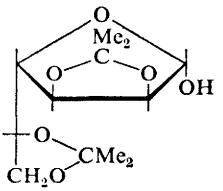
(38)



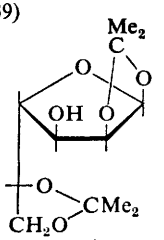
(39)



(40)



(41)



(42)

1,2:5,6-Di-*O*-isopropylidene-D-mannose, the only unknown 1,2:5,6-di-*O*-isopropylidene-D-hexose to date, has been prepared by reduction of the

[199] D. L. Ingles, *Austral. J. Chem.*, 1969, **22**, 1789.
[200] T. G. Bonner, E. J. Bourne, D. G. Gillies, and D. Lewis, *Carbohydrate Res.*, 1969, **9**, 463.
[201] J. D. Stevens, *Chem. Comm.*, 1969, 1140.

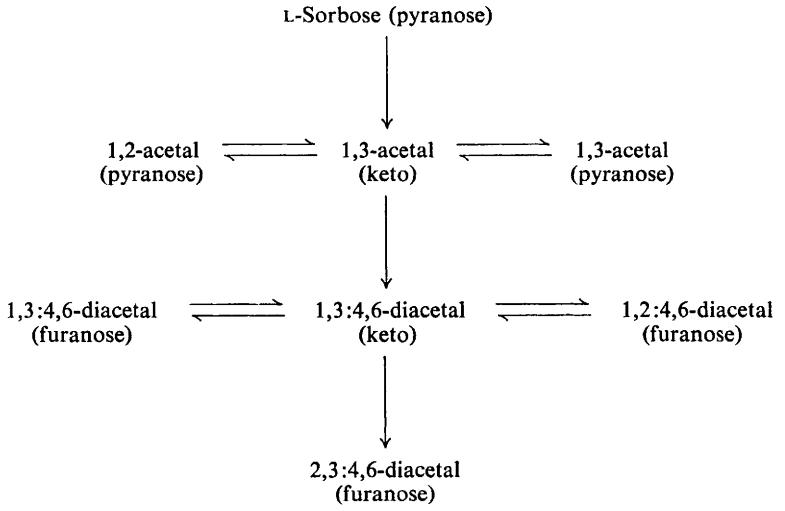
3-ketone derived from oxidation of the D-altrofuranose isomer. The intramolecular hydrogen bonding of the eight isomers of this series was examined and discussed, and the properties of the corresponding four ketones were investigated.[202] The major products of acetonation of α-D-talopyranose in the presence of anhydrous copper sulphate and sulphuric acid have been shown to be the 2,3:5,6-di-*O*-isopropylidene acetal (41, isolated in 28% yield), and the 1,2:5,6-isomer (42, 10%). The furanoid character of the products was established by mass spectrometry.[203]

Maeda and his group have continued to devote appreciable attention to L-sorbose and have reported on the complex reactions involved in the reaction of benzaldehyde with the sugar [204] (*cf.* vol. 1, p. 48; vol. 2, p. 41). Studies by the same group of workers on the structure of 1,3-*O*-benzylidene-L-sorbose by acetylation in pyridine, and by n.m.r., suggested that it existed in the furanose form in the crystalline state and as an equilibrium between furanose-, pyranose-, and keto-forms in solution.[205] A detailed paper has appeared, again from this group, on the n.m.r. spectra of isopropylidene derivatives of α-L-sorbose- and β-D-fructopyranose. For the former the introduction of an acetal ring at positions 1,2 or 1,3 did not affect the shape of the sugar ring, whereas a 2,3-acetal did. Di-*O*-isopropylidene-D-fructopyranoses having *cis*-fused rings also had distorted sugar ring conformations.[206] Further studies on the acetalation of L-sorbose have led to the conclusion given in Scheme 16.[207] N.m.r. has been used to assign the structure and conformation (1*C*) to 2,3:4,5-di-*O*-isopropylidene-β-D-fructose.[208]

A detailed study has been made of the ethylidenation of L-sorbose using acetaldehyde dimethylacetal in the presence of acid. After short reaction periods 1,3- and 1,2-α-furanoid products were obtained, but then the main product became the 1,3:4,6-β-L-furanose diacetal. This compound was transformed into two 2,3:4,6-α-furanoid diastereoisomers only under more acidic conditions.[209]

Oxalic acid has been shown to be a convenient hydrolysing agent for the selective removal of acetal rings of ketoses. It was noted that hydrolysis of 1,2-spiro acetal rings was much faster than those of other rings, and that this could be used as a means of identifying such spiro-systems.[210]

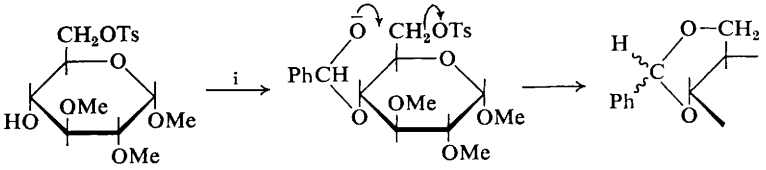
[202] K. N. Slessor and A. S. Tracey, *Canad. J. Chem.*, 1969, **47**, 3989.
[203] J. S. Brimacombe and P. A. Gent, *Carbohydrate Res.*, 1969, **9**, 231.
[204] T. Maeda, M. Kimoto, S. Wakahara, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1969, **42**, 1668.
[205] T. Maeda, M. Kimoto, S. Wakahara, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1969, **42**, 2021.
[206] T. Maeda, K. Tori, S. Satoh, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1969, **42**, 2634.
[207] T. Maeda, Y. Miichi, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1969, **42**, 2648.
[208] V. I. Glazkov, E. M. Malakhaev, and B. N. Stepanenko, *Doklady Akad. Nauk S.S.S.R.*, 1968, **182**, 1322.
[209] T. Maeda, M. Kiyokawa, and K. Tokuyama, *Bull. Chem. Soc. Japan*, 1969, **42**, 492.
[210] R. S. Tipson, B. F. West, and R. F. Brady jun., *Carbohydrate Res.*, 1969, **10**, 181.



Scheme 16

Modified procedures have been described for the isolation of 2,3-*O*-isopropylidene-β-D-*threo*-pentulofuranose and 1,2:3,4-di-*O*-isopropylidene-D-*erythro*-pentulofuranose after pyridine-catalysed isomerisations of D-xylose and D-arabinose, respectively. This procedure provided a method for obtaining the pentuloses, several derivatives of which were described.[19] A new di-*O*-isopropylidene-D-apiose is mentioned on p. 123.

(*ii*) *Glycosides, etc.* A new method for preparing acetals under kinetic control has been investigated, but because of the moderate yields obtained it was not considered to represent an important improvement on available methods. The principle is illustrated in Scheme 17.[211]

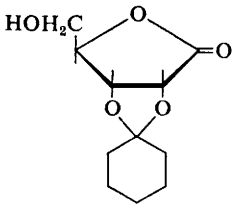


Reagents: i, PhCHO, Bu^{t}OK, BuOH

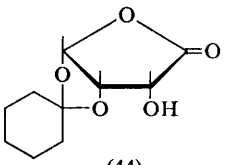
Scheme 17

Treatment of D-ribono-1,4-lactone with cyclohexanone in the presence of an acidic resin gave two products designated as (43) and (44) (obtained in 60 and 20% yield respectively) which were isolated by fractional crystallisation.[212]

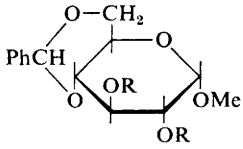
[211] N. Baggett, M. D. Mosiuzzaman, and J. M. Webber, *Carbohydrate Res.*, 1969, **11**, 263.
[212] A.-M. Supulchre, A. Gateau, and S. D. Géro, *Compt. rend.*, 1969, **269**, *C*, 1312.



(43)



(44)



R = CH(Me)OEt

(45)

From Single Alcoholic Groups.—Polyacetals, each formed from one hydroxy-group of sugar derivatives, have been reported. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside on treatment in benzene solution with ethyl vinyl ether in the presence of toluene-*p*-sulphonic acid afforded the diacetal (45). From D-glucose a fully substituted syrupy product was obtained with methyl vinyl ether in DMF, and methyl β-D-arabinopyranoside with dihydropyran gave a crystalline trisubstituted tetrahydropyranyl derivative. The ethoxyethyl substituents in compound (45) were removed with mild acid under conditions which did not affect the benzylidene ring.[213]

A series of alkoxymethyl ethers of sucrose having long-chain alkyl groups have been synthesised and examined.[214]

[213] M. L. Wolfrom, S. S. Bhattacharjee, and R. M. De Lederkremer, *Carbohydrate Res.*, 1969, **11**, 148.

[214] V. A. Lutsenko, O. P. Koren'kova, and R. M. Panich, *Neftepererab. Neftekhim.*, 1968, 31 (*Chem. Abs.*, 1969, **71**, 30,640*s*).

6
Esters

Acetates

The four methyl α-D-glucopyranoside tetra-acetates, each bearing one trideuteriated ester group, have been synthesised by specific routes, and have been used to assign the individual acetyl resonances in the n.m.r. spectrum of the compound in various solvents. Similarly, the acetyl resonances in the spectrum of methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside were assigned. With this information it was possible to analyse accurately the products of partial acetylation of methyl α-D-glucopyranoside and its 4,6-*O*-benzylidene derivative by using deuteriated acetylating agents and following the initial esterification by complete acetylation with unlabelled reagents. When 1 mole of acetic anhydride had reacted with the glycoside in pyridine solution C-6, C-4, C-3, and C-2 substitutions were found to have occurred in the ratio 40 : 20 : 20 : 20. With the benzylidene compound, when 0·67 mole of acetyl group had been incorporated the ratio of acetates was C-3, 0·37 : C-2, 0·30.[215] Appreciable new information on acetates, derived by n.m.r. methods, is referred to in Chapter 23.

The problem of locating the ester groups in partially acetylated sugars by chemical means has been considered in detail, the principle being to find protecting derivatives to mask the free hydroxy-groups, followed by removal of the acetates and location of the new hydroxy-groups by standard methylation techniques. A wide range of blocking groups, including TMS, alkyl, aryl, allyl, vinyl, and tetrahydropyranyl ethers, carbonates, thiocarbonates, formates, trifluoroacetates, benzoyl, and phenylcarbamoyl esters, was assessed and all were found to be unsuited to this particular purpose. Finally, nitrate esters, formed at 0°C with nitric acid in acetic anhydride, were found to be satisfactory since they could be introduced without acetyl migration and were stable during subsequent deacetylations and methylations.[216]

2,3-Di-*O*-acetyl-D-glucopyranose has been synthesised (by hydrogenolysis of benzyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside) as part of a study of the enzymic substrate properties of partially acetylated

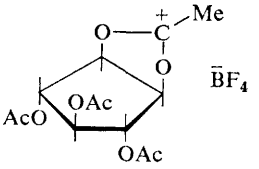
[215] D. Horton and J. H. Lauterbach, *J. Org. Chem.*, 1969, **34**, 86.
[216] A. L. Fink and G. W. Hay, *Canad. J. Chem.*, 1969, **47**, 845.

glucoses. The compound was characterised by conversion into 2,3-di-*O*-methyl-D-glucose and was found to be surprisingly labile and readily deacetylated.[217]

Heating methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and -β-D-galactopyranoside in anhydrous labelled acetic acid caused isotopic incorporation without optical rotational change. The ester groups therefore underwent solvolysis, but the anomeric centre was stable under these conditions.[218]

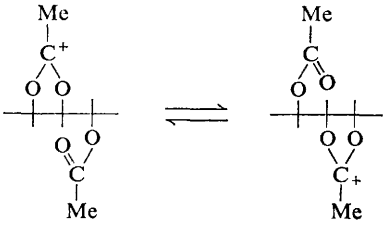
Deacetylations can be effected enzymically, and ester groups have been removed from mono- and di-saccharide acetates with wheatgerm lipase and with a purified esterase obtained from it. For peracetylated glucose, the order of de-esterification reactivity is C-1 > C-6 > C-4 > C-3 > C-2. Acetates were removed twelve times more readily than propionyl esters, and twenty-five times more readily than benzoates.[219]

Paulsen and his colleagues have continued their researches on acetoxonium ions and have prepared the interesting tetrafluoroborate (46), which



(46)

underwent rapid and continuous cyclorearrangements with the ion moving round the ring.[220] Acetoxonium salts of simple systems have also been examined. Diol diacetates and triol triacetates (both cyclic and acyclic) on treatment with antimony pentachloride in methylene dichloride give these salts. In the case of systems such as (47), two n.m.r. signals due to methyl groups were observed at room temperature but at elevated temperatures rapid reversible rearrangement occurred and the signals coalesced (Scheme 18).[221]



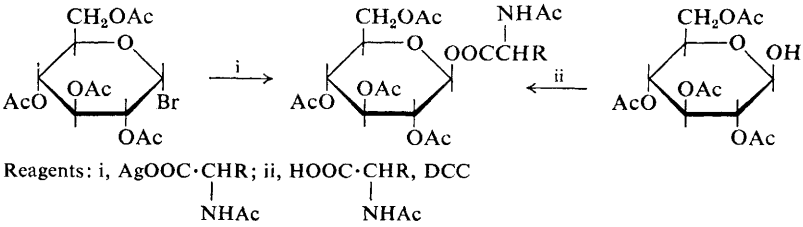
(47)

Scheme 18

[217] A. L. Fink and G. W. Hay, *Canad. J. Chem.*, 1969, **47**, 841.
[218] J. Strucinski and J. Świderski, *Roczniki Chem.*, 1969, **43**, 1236.
[219] A. L. Fink and G. W. Hay, *Canad. J. Biochem.*, 1969, **47**, 353.
[220] H. Paulsen and H. Behre, *Angew. Chem. Internat. Edn.*, 1969, **8**, 887.
[221] H. Paulsen and H. Behre, *Angew. Chem. Internat. Edn.*, 1969, **8**, 886.

Substituted Acetates and Other Nonaromatic Carboxylates

Several unusual glycosyl esters have been prepared. Amino-acid derivatives have been synthesised by two routes (Scheme 19) as models for the carbohydrate–protein branch points in glycoproteins.[222] The synthesis of

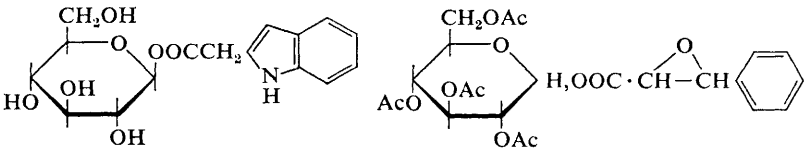


Reagents: i, $AgOOC{\cdot}\underset{\displaystyle NHAc}{\underset{|}{CHR}}$; ii, $HOOC{\cdot}\underset{\displaystyle NHAc}{\underset{|}{CHR}}$, DCC

R = H, Me, Et, $MeSCH_2CH_2$, or Bn

Scheme 19

1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (48) from tetra-*O*-benzyl-D-glucopyranosyl chloride and the silver salt of the acid has been reported. The growth-promoting effect of the ester was distinctly greater than the combined effect of the acid and unbound glucose. The compound has also been isolated from intact *Avena* coleoptiles.[223] The related esters (49) were



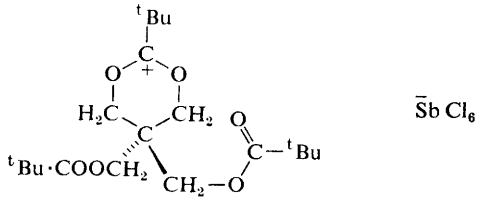
(48) (49)

also synthesised and found to react with nucleophiles at the non-benzylic epoxide carbon atom; amino- and alkoxy-derivatives were thus obtained.[224]

β-Benzoylpropionyl groups have been recommended as hydroxy-group-protecting derivatives in nucleoside and oligonucleotide syntheses. They have the advantage of being removed with dilute hydrazine hydrate in pyridine–acetic acid solutions which are effectively neutral.[225] Trifluoroacetates have been recommended for the g.l.c. resolution of alditols (see Chapter 26),[226] and reference is made to the ^{19}F n.m.r. of these esters in Chapter 23.

Di-*O*-pivaloylpentaerythritol-*O*-pivaloxonium hexachloroantimonate (50) undergoes a cyclorearrangement of the type reported for compound (46).[227]

[222] A. Kornhauser and D. Keglevic, *Carbohydrate Res.*, 1969, **11**, 407.
[223] D. Keglevic and M. Pokorny, *Biochem. J.*, 1969, **114**, 827.
[224] A. I. Nogaldeli and R. A. Gakhokidze, *Soobshch. Akad. Nauk. Gruz, S.S.R.*, 1969, **53**, 85 (*Chem. Abs.*, 1969, **71**, 30,663*b*).
[225] R. L. Letsinger and P. S. Miller, *J. Amer. Chem. Soc.*, 1969, **91**, 3356.
[226] J. Shapira, *Nature*, 1969, **222**, 792.
[227] H. Paulsen, H. Meybourg, and H. Behre, *Angew Chem. Internat. Edn.*, 1969, **8**, 888.



(50)

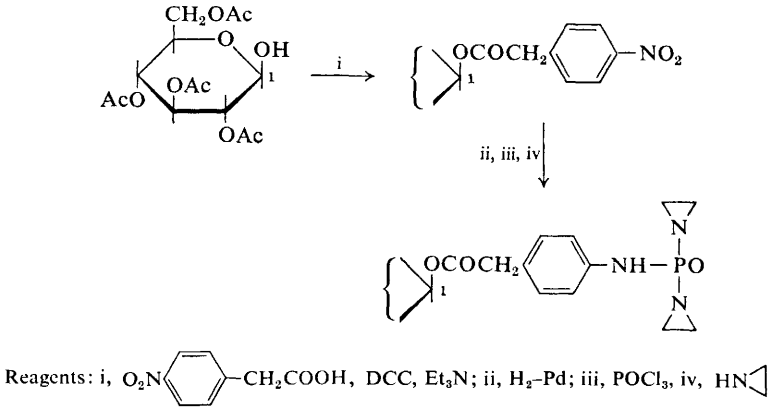
Various new high-molecular-weight esters have been reported. Polymers of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose 6-methacrylate and 6-acrylate have been synthesised,[172] and five sucrose monopalmitates were separated and structurally analysed by n.m.r. methods. Esterification was shown to have occurred predominantly at positions 6 and 6′ and not at all at position 1′.[228] Other workers have drawn the same conclusions regarding the relative reactivities of these hydroxy-groups from studies of acylations using long-chain fatty acid derivatives. Several crystalline mono- and di-esters were obtained.[229] Related crystalline esters bearing substituents at the primary hydroxy-groups were prepared from D-mannitol.[230]

Benzoates

The selective 3-*O*-benzoylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside and subsequent alkali-induced migration of the benzoyl group to C-2 have been reported. Under similar conditions the 3-*O*-benzoate of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside may be formed from the 2-benzoate. Under equilibrium conditions it was shown that both the 2- and 3-benzoates were present in the glucose and the galactose series.[231]

By use of ^{14}C-labelled benzoyl groups, a study has been made of the contribution of the various benzoate groups of glucose and galactose benzoates to the formation of the 1,1-bis(benzoylamido)-1-deoxyalditols produced by ammonolysis. It was found that, (i) the benzoyl group at C-1 did not contribute; (ii) in acyclic aldehydo-benzoates the ester group at C-2 contributed to a high degree (0·81 mole/mole); (iii) for furanose and pyranose benzoates the C-2 ester contribution was small (0·12 mole/mole); (iv) for furanose benzoates the benzoyl group at O-3 contributed extensively (0·8 mole/mole for glucose and 0·62 for galactose); (v) the O-4 ester contributed 0·81 for glucose and 1·0 for galactose, and (vi) the O-6 ester contributed 0·30 for D-glucopyranose pentabenzoate and 0·18 for the *galacto*-isomer. All the results were discussed in detail.[232] Ethyleneimino-groups have been introduced into glycosyl benzoates as shown in Scheme 20.[233]

[228] T. Otake and E. Tamate, *J. Chem. Soc. Japan*, 1969, **90**, 393.
[229] E. Reinefeld and S. Klaudianos, *Zucker*, 1968, **21**, 330.
[230] E. Reinefeld and G. Klauenberg, *Tenside*, 1968, **5**, 266.
[231] G. J. F. Chittenden and J. G. Buchanan, *Carbohydrate Res.*, 1969, **11**, 379.
[232] A. B. Zanlungo, J. O. Deferrari, and R. A. Cadenas, *Carbohydrate Res.*, 1969, **10**, 403.
[233] D. Dziuviene and J. Degutis, *Zhur. obshchei Khim.*, 1969, **39**, 83.



Reagents: i, O_2N–CH_2COOH, DCC, Et_3N; ii, H_2–Pd; iii, $POCl_3$, iv, HN

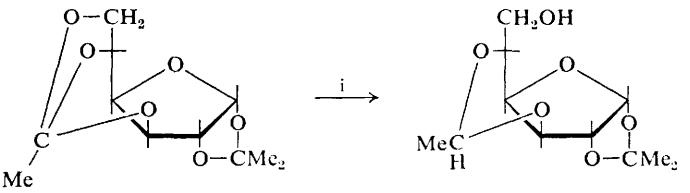
Scheme 20

The structure of hamamelitannin as the 1,5-digalloyl ester of 2-*C*-hydroxymethyl-D-ribofuranose has been confirmed by synthesis.[234]

The chirality of *cis*- and *trans*-glycols can be determined by examination of the o.r.d. or c.d. curves given by the derived benzoates (see p. 182).

Carboxylic Orthoesters

Orthoesters, on treatment with lithium aluminium hydride and aluminium chloride, gave acetals selectively, since this reaction proceeded faster than the reductive ring-opening of the products (*cf.* p. 30). Acetals may therefore be obtained by this process, as is illustrated in Scheme 21.[168]



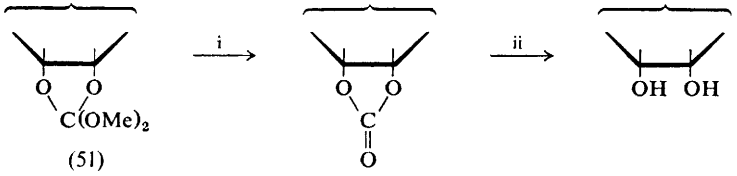
Reagent: i, LAH–$AlCl_3$

Scheme 21

Carbonates

Ribonucleosides have been made to undergo acid-catalysed exchange with tetramethyl orthocarbonate [$C(OMe)_4$] to give 2′,3′-*O*-dimethoxymethylidene derivatives (51). These are potentially generally useful protecting-derivatives since they are alkali stable but with mild aqueous acid give cyclic carbonates (Scheme 22), which may then be removed with bases.[234a]

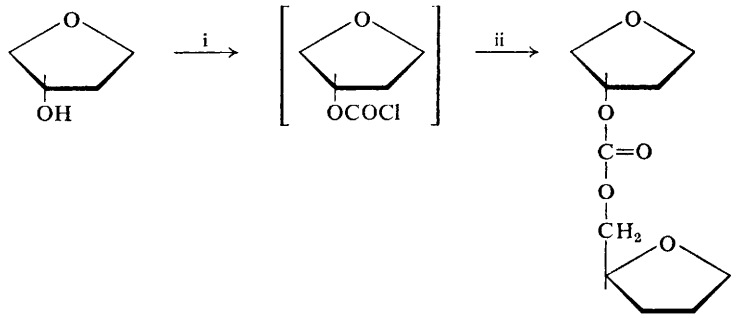
[234] A. D. Ezekiel, W. G. Overend, and N. R. Williams, *Carbohydrate Res.*, 1969, **11**, 233.
[234a] G. R. Niaz and C. B. Reese, *Chem. Comm.*, 1969, 552.

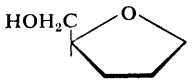


Reagents: i, H^+; ii, ^-OH

Scheme 22

'Dinucleotides' have now been prepared with carbonate diester linkages joining the nucleosides; two methods have been reported for effecting such syntheses; (i) the use of the $\beta\beta\beta$-trichloroethoxycarbonyl ester group attached to one nucleoside which is condensed with monohydroxynucleoside derivatives,[235] and (ii) similar use of the chloroformate group introduced with phosgene (as outlined schematically in Scheme 23).[236]



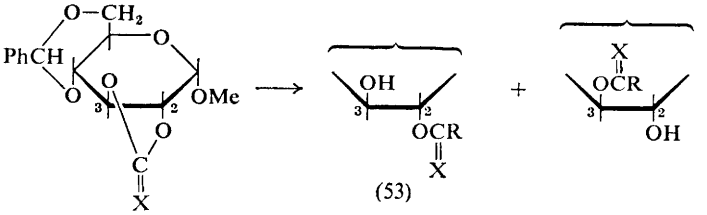
Reagents: i, $COCl_2$; ii, 

Scheme 23

The glucopyranoside 2,3-carbonate (52, X = O) reacted readily with methanol, benzyl alcohol, α-toluenethiol, ammonia, piperidine, and glycine methyl ester to give the corresponding 2-*O*- and 3-*O*-carbonate derivatives (53, X = O) with the 2-isomers preponderating (Scheme 24).[237]

Cyclic carbonates have been used as intermediates, prepared from thiocarbonates, for effecting specific stereochemical inversions as illustrated in Scheme 25. Sulphonated compounds or halogeno-derivatives may be used to provide the leaving group.[238]

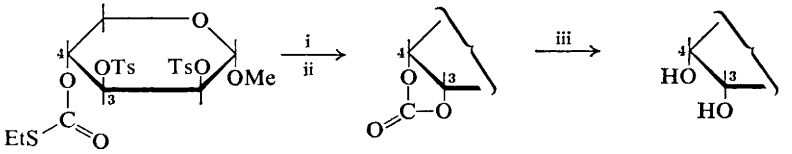
[235] D. S. Jones and J. R. Tittensor, *Chem. Comm.*, 1969, 1240.
[236] M. P. Mertes and E. A. Coats, *J. Medicin. Chem.*, 1969, **12**, 154.
[237] W. M. Doane, B. S. Shasha, E. I. Stout, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1969, **11**, 321.
[238] E. J. Reist and S. H. Cruse, *Carbohydrate Res.*, 1969, **10**, 289.



(52) (53)

R = OMe, OBn, SBn, NH_2, NHPh, or $NHCH_2COOMe$

Scheme 24



Reagents: i, NaF, DMF; ii, H_2O; iii, MeO^-

Scheme 25

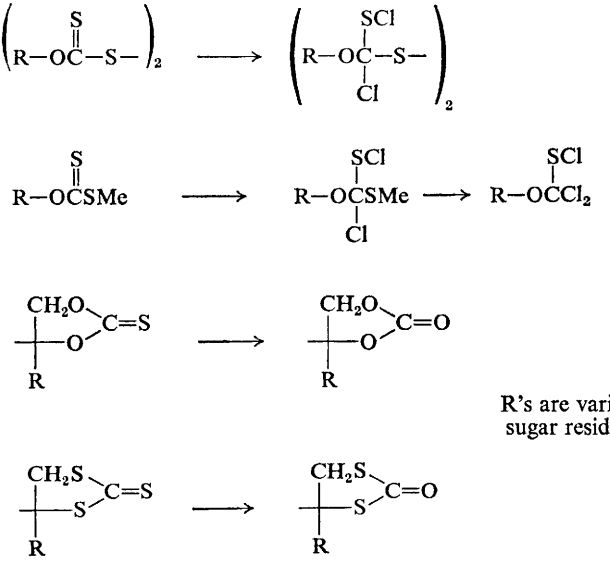
Thiocarbonates

The use of thiocarbonates to effect configurational inversions is illustrated in Scheme 25, and the reactions indicated in Scheme 24 proceeded in the same way when thionocarbonates were involved (52, 53, X = S).[238]

The reactions of several thiocarbonyl carbohydrate derivatives with chlorine were examined; the findings are outlined in Scheme 26.[239]

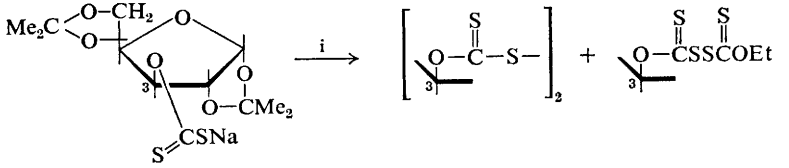
The distribution of xanthate groups introduced on partial xanthation of methyl 4-*O*-methyl-β-D-glucopyranoside has been determined, and the relative reactivities of the hydroxy-groups were found to decrease in the order C-6 > C-3 > C-2. After treatment with alkali the ester mixtures contained more 6-derivative and negligible amounts of the 2-isomer.[240] A series of benzylxanthate esters of methyl α-D-glucopyranoside selectively methylated at various positions were then prepared, and the behaviour of the derived xanthate salts in alkaline media was studied. The results revealed that migration of the ester groups from positions 2 and 3 to position 6 proceeded by way of position 4. Direct migration across the pyranose ring was negligible.[241]

[239] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *J. Org. Chem.*, 1969, **34**, 1642.
[240] P. J. Garegg and K. Lindstrom, *Svensk Papperstidn.*, 1969, **72**, 421.
[241] D. Trimnell, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1969, **11**, 497.



Scheme 26

Unsymmetrical dithiobis(thioformates) have been prepared as shown in Scheme 27, and their disproportionation reactions to xanthates and cyclic thionocarbonates have been studied.[242]



Reagents: i, EtOC(S)SNa, I_2

Scheme 27

By condensations between acylated glycosyl halides and sodium dimethyldithiocarbamate ($Me_2NCS.SNa$), glycosyl thioester derivatives have been produced which had antifungal and antibacterial properties.[108]

Cyclic thionocarbonates can be used to determine the chiralities of vicinal diols by o.r.d. methods (see p. 182).

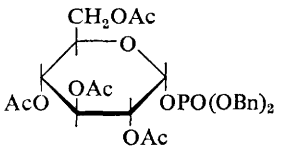
Phosphates and Phosphites

Carbohydrate Phosphates and Phosphites.—Because of its potential significance from the standpoint of prebiological chemistry, the phosphorylation of D-ribose in aqueous solution in the presence of cyanogen or cyanamide

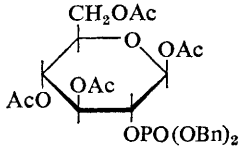
[242] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1969, **10**, 449.

has been studied. β-D-Ribofuranosyl phosphate was the only product found. 2-Deoxy-D-*erythro*-pentose was not phosphorylated under the same conditions.[243]

β-D-Glucopyranosyl phosphate has been synthesised by debenzylation and deacetylation of the product from the stereospecific reaction occurring between 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose and dibenzyl phosphate.[244] In related work by the same authors, the reaction between 3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-β-D-glucopyranosyl chloride and silver dibenzyl phosphate gave the α-phosphate (54). During selective



(54)



(55)

removal of the C-2 substituent from this compound a phosphate migration occurred, and the product afforded, after acetylation, was (55). However, debenzylation of the initial product (54), followed by deacetylation, gave α-D-glucopyranosyl phosphate.[245]

α-L-Fucose 1-phosphate has been prepared *via* the acetylated α-glycosyl chloride and compared with the biochemically synthesised β-anomer. It was suggested that the initial product was the β-ester and that it anomerised under the conditions of the reaction,[246] but a mechanism involving a double inversion at C-1 and incorporating neighbouring-group participation by the C-2 acetoxy-group would seem more likely.

Phosphorylation of *o*-nitrophenyl β-D-galactopyranoside with phosphorus oxychloride in trimethyl phosphate affords an improved method for the preparation of the 6-phosphate.[247] The phosphorylation of 5,6-*O*-isopropylidene ascorbic acid is referred to on p. 141.

The acid-catalysed hydrolysis and spontaneous hydrolysis of α-D-ribofuranosyl phosphate and α-D-glucopyranosyl phosphate have been compared, and the former found to be several hundred times more reactive in both reactions. The catalytic effects of different mineral acids were compared, and salt effects were examined. The factors influencing the rates of hydrolysis of furanosyl and pyranosyl phosphates (and furanosides and pyranosides) were considered in detail. Steric, dipolar, and entropy factors were all believed to contribute to the reactivities.[248]

[243] M. Halmann, R. A. Sanchez, and L. E. Orgel, *J. Org. Chem.*, 1969, **34**, 3702.
[244] C. L. Stevens and R. E. Harmon, *Carbohydrate Res.*, 1969, **11**, 99.
[245] C. L. Stevens and R. E. Harmon, *Carbohydrate Res.*, 1969, **11**, 93.
[246] D. H. Leaback, E. C. Heath, and S. Roseman, *Biochemistry*, 1969, **8**, 1351.
[247] W. Hengstenberg and M. L. Morse, *Carbohydrate Res.*, 1969, **10**, 463.
[248] C. A. Bunton and E. Humeres, *J. Org. Chem.*, 1969, **34**, 572.

1,2:3,4:5,6-Cyclic alkyl phosphite derivatives of D-mannitol have been reported,[249] and heating triethyl phosphite with methyl α-D-glucopyranoside gave the 4,6-cyclic ethyl phosphite 2-diethylphosphite and a further ester.[250]

Nucleoside Phosphates.—Specific phosphorylations at the 5′-positions of thymidine and uridine were effected using diethyl azodicarboxylate, triphenylphosphine, and dibenzyl hydrogen phosphate, a set of reagents which yields dibenzyl phosphate esters with alcohols. The benzyl groups were then removed by hydrogenolysis.[251] Direct phosphorylation, using pyrophosphoryl chloride in *m*- or *o*-chlorophenol, of a variety of natural and synthetic purine and pyrimidine nucleosides also resulted in the selective phosphorylation of the primary hydroxy-groups.[252] Specific 5′-phosphorylation of 9-β-D-arabinofuranosyl-9*H*-purine-6(1*H*)-thione has been achieved following 5′-tritylation.[253] By the phosphorylation of known 5′-amino-5′-deoxynucleoside derivatives and their 2′-deoxy-analogues, using various phosphorylating reagents such as bis(βββ-trichloroethyl)phosphorochloridate, nucleotide analogues having 5′-amino groups were synthesised, and thence thymidyl-(3′,5′)-5′-amino-5′-deoxy-thymidine having an internucleotide P—N bond was prepared as a model dinucleotide.[254]

The synthesis of the 3′,5′-cyclic phosphate of 9-(β-D-xylofuranosyl)-adenine has been described.[255]

The action of hydrogen fluoride on nucleotides has been examined. In the majority of cases reactions and conditions can be controlled so that dephosphorylation is the only reaction which occurs. The reaction of 2′-deoxyadenosine 5′-phosphate was in marked contrast; cleavage of the glycosylamine bond was the predominant reaction.[256]

Russian workers have synthesised a series of sugar nucleotides using glycosyl bromides and silver dibenzyl phosphate. Hydrogenolysis of the products gave pyrophosphates, which were condensed with nucleoside 5′-phosphormorpholides. The following products were prepared and their biological properties were studied: UDP-6-deoxy-D-glucose,[257] UDP-4-deoxy-D-glucose,[258] UDP-3-deoxy-D-glucose,[259] N_1-methyl

[249] O. V. Voskresenskaya, N. A. Makarova, P. A. Kirpichnikov, and E. T. Mukmenev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1626.
[250] O. V. Voskresenskaya, N. A. Makarova, P. A. Kirpichnikov, and E. T. Mukmenev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1624.
[251] O. Mitsunobu, K. Kato, and J. Kimura, *J. Amer. Chem. Soc.*, 1969, **91**, 6510.
[252] K. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, 1969, **34**, 1547.
[253] J. P. Bell, *Canad. J. Chem.*, 1969, **47**, 1095.
[254] B. Jastorff and H. Hettler, *Chem. Ber.*, 1969, **102**, 4119.
[255] M. Hubert-Habart and L. Goodman, *Chem. Comm.*, 1969, 740.
[256] D. Lipkin, B. E. Phillips, and J. W. Abrell, *J. Org. Chem.*, 1969, **34**, 1539.
[257] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and J. J. Kusov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1136.
[258] N. K. Kochetkov, E. I. Budowsky, T. N. Druzhinina, N. D. Gabrielyan, and J. J. Kusov, *Carbohydrate Res.*, 1969, **10**, 152.
[259] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, J. J. Kusov, and I. V. Komlev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 2522.

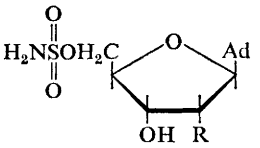
ADPG,[259a] and N_6-methyl and N_6N_6-dimethyl ADPG.[260] Also, the inosine, xanthosine, and 2′-deoxyadenosine analogues of ADPG were synthesised.[260]

An independent study of the optimal conditions for the synthesis of the glycosyl pyrophosphates used in the above preparations was undertaken.[261]

Sulphates

The sulphation of sugar derivatives with the pyridine–sulphur trioxide reagent has been shown to be an equilibrium reaction. The preparations of a number of barium salts of sugar and glycoside disulphates have been described, and β-D-galactose 4-sulphate and α- and β-D-galactoside 4-sulphates have been synthesised by improved methods.[262] The specific synthesis of D-mannose 6-sulphate has been developed and involved the substitution, using the pyridine–sulphur trioxide reagent, of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose followed immediately by deacetylation with methanolic ammonia.[263]

Compounds (56) and (57) have been synthesised by use of suitably substituted adenosine derivatives and sulphamoyl chloride. These are structural models of nucleocidin (p. 65) but lacking fluorine, and the former can be viewed as a nonionic analogue of adenosine 5′-phosphate. It was found to show pronounced antibacterial activity.[264]



(56) R = OH
(57) R = H

Chlorosulphates are mentioned on p. 173 and several fluorosulphates have been prepared by halide exchange reactions, using silver fluoride, from chlorosulphates.[265]

Sulphonates

The second part of a two-part review on sulphonates has been published.[265a] (*cf.* vol. 2, p. 60).

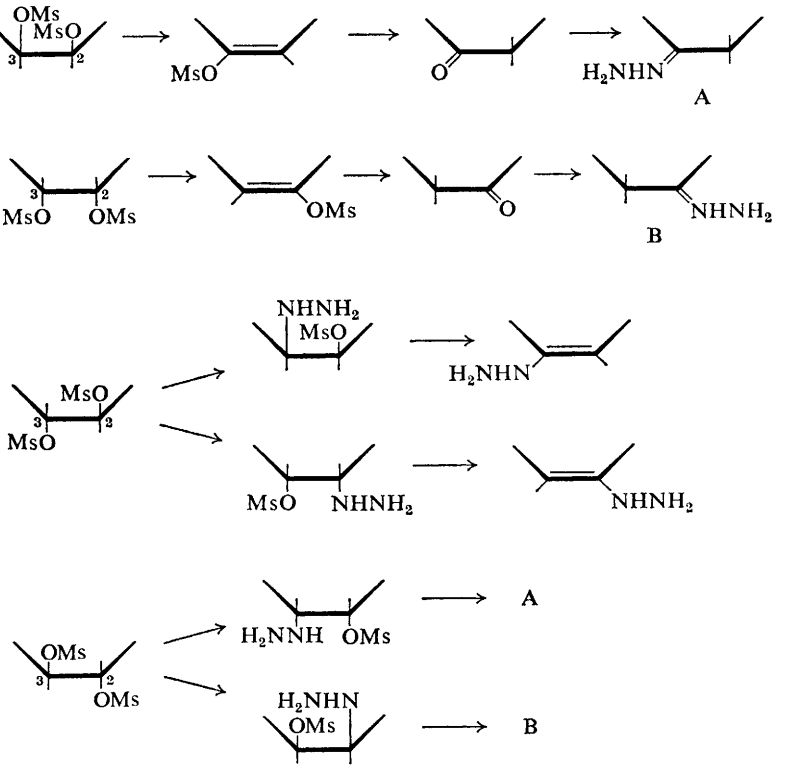
A new method for the removal of toluene-*p*-sulphonyl groups has been described, in which primary and secondary esters were photolysed at room

[259a] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and S. M. Spiridonova, *Khim. prirod. Soedinenii*, 1968, 123.
[260] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and S. M. Spiridonova, *Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1969, 2514, 2775.
[261] N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, and K. S. Lebedeva, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 897.
[262] M. J. Harris and J. R. Turvey, *Carbohydrate Res.*, 1969, **9**, 397.
[263] P. F. Lloyd and C. H. Stuart, *Carbohydrate Res.*, 1969, **10**, 174.
[264] D. A. Shuman, R. K. Robins, and M. J. Robins, *J. Amer. Chem. Soc.*, 1969, **91**, 3391.
[265] E. Buncel, H. J. Jennings, J. K. N. Jones, and I. M. E. Thiel, *Carbohydrate Res.*, 1969, **10**, 331.
[265a] D. H. Ball and F. W. Parrish, *Adv. Carbohydrate Chem.*, 1969, **24**, 139.

temperature in methanolic sodium methoxide.[266] Glycoside and isopropylidene groups were not affected under these conditions.

Displacement Reactions without Participation.—Direct displacements with azide ion have been used in the synthesis of amino-sugars and will not be referred to here (see Chapter 8). Richardson has summarised his views on displacement reactions of sulphonate esters and has explained the diminished reactivity in S_N2 displacements of aldopyranoside 2-esters, hexulopyranose 1-esters, and 6-derivatives in the galactose series by considering the nature of the highly polar transition state [267, 268] (*cf.* vol. 2, p. 61).

The normally difficult-to-displace toluene-*p*-sulphonyloxy-group in the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-ester has been successfully displaced by thiolacetate ion.[269]



all are methyl 4,6-*O*-benzylidene-α-D-glycosides

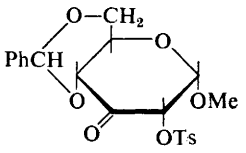
Scheme 28

[266] S. Zen, S. Tashima, and S. Koto, *Bull. Chem. Soc. Japan*, 1968, **41**, 3025.
[267] A. C. Richardson, *Carbohydrate Res.*, 1969, **10**, 395.
[268] Y. Ali and A. C. Richardson, *J. Chem. Soc.* (*C*), 1969, 320.
[269] U. G. Nayak and R. L. Whistler, *Chem. Comm.*, 1969, 434.

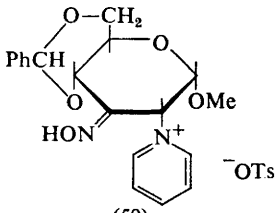
The formation of epoxides from various mono- and di-toluene-*p*-sulphonyl derivatives of 1,6-anhydro-β-D-glucopyranose has been studied.[180]

The reactions of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methanesulphonyl-D-hexopyranosides with hydrazine have been studied, and the findings are summarised in Scheme 28. Eliminations were followed by ketonisation in each case, and the products obtained were governed by the ease of the initial elimination or substitution.[270] For further studies on the hydrazone products see p. 80. Reactions of sulphonates leading to unsaturated products are described on pp. 116—119. Related displacement reactions of halogeno-derivatives are discussed on p. 66.

Treatment of the keto-sugar (58) with hydroxylamine hydrochloride in aqueous pyridine gave a 91% yield of the pyridinium salt (59), which underwent ready nucleophilic displacements at C-2.[271]



(58)



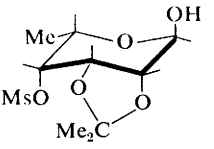
(59)

Displacements with Participation.—(i) *Oxygen functions.* Full details have appeared of work reported previously in preliminary form, but covered in earlier Reports. These are the solvolysis of methyl 6-*O*-methanesulphonyl-2,3-di-*O*-methyl-β-D-galactopyranoside (methoxy-group participation)[272] (see vol. 2, p. 70) and of benzyl 5-*O*-*p*-bromobenzenesulphonyl-2,3-*O*-β-D-ribofuranoside (benzyloxy-group participation)[273] (see vol. 2, p. 69). Full details have also been given of the reaction of 1,6-anhydro-3,4-*O*-isopropylidene-2-*O*-methanesulphonyl-β-D-galactopyranoside with potassium fluoride dihydrate in methanol (see vol. 1, p. 74).[274]

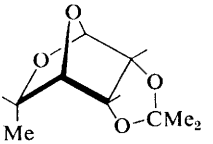
The use of the (ethanethio)carbonyl group in participation reactions has been noted.[238]

The solvolysis of 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranose (60) led to the formation of three products (61), (62), and (63) in yields of 56, 26, and 12% respectively. The competing factors involved in the mechanisms were discussed.[275]

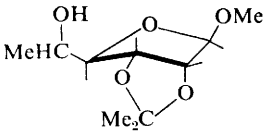
[270] H. Paulsen and D. Stoye, *Chem. Ber.*, 1969, **102**, 834.
[271] W. A. Szarek, B. T. Lawton, and J. K. N. Jones, *Tetrahedron Letters*, 1969, 4867.
[272] J. S. Brimacombe and O. A. Ching, *Carbohydrate Res.*, 1969, **9**, 287.
[273] J. S. Brimacombe and O. A. Ching, *J. Chem. Soc.* (*C*), 1969, 964.
[274] N. A. Hughes, *J. Chem. Soc.* (*C*), 1969, 2263.
[275] J. S. Brimacombe, F. Hunedy, and A. K. Al-Radhi, *Carbohydrate Res.*, 1969, **11**, 331.



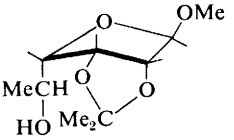
(60)



(61)

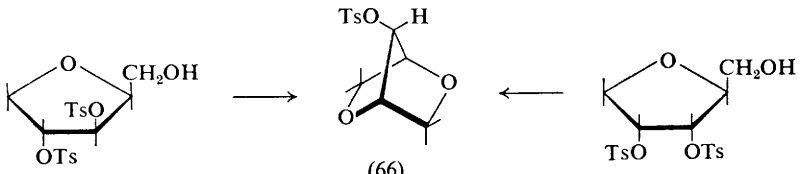


(62)



(63)

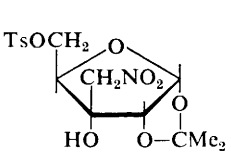
Treatment of either of the 2,5-anhydro-pentitol derivatives (64) or (65) with methanolic sodium methoxide gave the 2,5-dioxabicyclo[2,2,1]heptane derivative (66).[276]



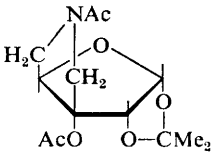
(64) (66) (65)

(ii) *Nitrogen functions.* Participation by nitrogen functions has led to the synthesis of epimines and oxazolines; see Chapter 10, pp. 90 and 93, respectively. The use of acetamido neighbouring-groups to produce *cis*-hydroxy-acetamido-systems from *trans*-precursors continues to be a useful reaction; see pp. 70, 71.

Reduction of the nitro-compound (67) followed by acetylation gave the bicyclic derivative (68).[277]



(67)

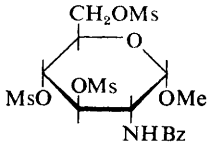


(68)

Reaction of the tri-*O*-methanesulphonyl-*gluco*-derivative (69) with sodium azide in HMPT for 24 hr gave the *galacto*-derivative (70), *i.e.* there had been retention of configuration at C-3. It was presumed that the reaction occurred *via* the oxazolinium azide which then rearranged to give

[276] J. Cléophax, S. D. Géro, A. Gaudemer, and A. M. Sépulchre, *Carbohydrate Res.*, 1969, **9**, 361.

[277] G. J. Lourens, *Tetrahedron Letters*, 1969, 3733.



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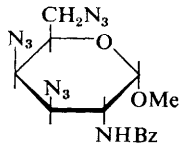
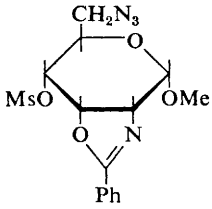
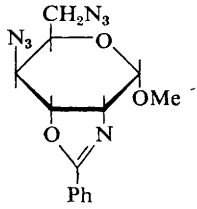


(70)

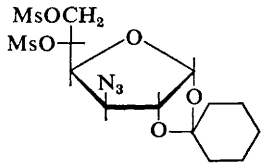


(71)

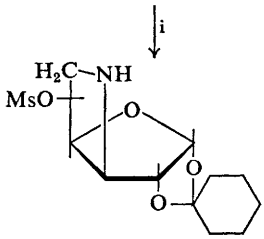


(72)

(70). Brief treatment of (69) with sodium azide, followed by sodium acetate in aqueous 2-methoxyethanol, gave (71), which on further treatment with azide ion gave the oxazoline (72). Reaction of this compound with azide

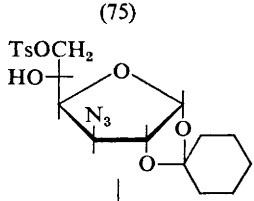


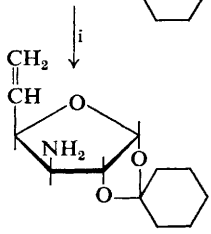
(73)



(74)

(75)





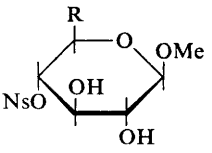
Reagents: i, Zn, NaI, DMF

Scheme 29

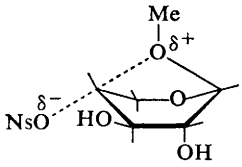
ion did not give (70), showing that it was not an intermediate in the direct transformation of (69) to (70).[268]

Consideration of the two reactions shown in Scheme 29 and analysis of possible reaction pathways led to the postulation of neighbouring-group participation by azide or by complexed azide for the reactions (73) to (74), followed by reduction. Chelation of the hydroxy- and azido-groups with zinc in (75) was thought to prevent the above reaction and thus allow the normal elimination reaction to occur.[278]

Reaction of Nitrobenzene-*p*-sulphonates.—Solvolysis of methyl 4-*O*-nitrobenzene-*p*-sulphonyl-β-D-xylopyranoside (76) gave the 3,4-epoxide (40%), methyl α-L-arabinopyranoside (by inversion at C-4) (15%), and 4-*O*-methyl-L-arabinose (40%). Similar results have been obtained with the glucose analogue (77) of (76) (see vol. 1, p. 80). The 4-*O*-methyl-L-arabinose was presumed to have arisen *via* the boat-like transition state (78).[279]



(76) R = H
(77) R = CH_2OH



(78)

Nitrates and Nitrites

Little new work has been reported on nitrates, but these esters were found to be useful in a new technique for locating the position of acetyl groups in partially acetylated compounds.[216] Nitrites of 1,4:3,6-dianhydrohexitols are mentioned on p. 182.

Borates, Borinates, and Boronates

Heating nucleosides with equimolar amounts of isobutyl diphenylborinate in pyridine gave 2′,3′-phenylboronates, which were also prepared directly by use of phenylboronic acid.[280] Phenylborinic acid has again been used as a protecting group in synthetic work (p. 29), and a xylosylamine 2,4-phenylboronate has been subjected to *X*-ray-crystallographic analysis.[281]

[278] H. Ohrui and S. Emoto, *Carbohydrate Res.*, 1969, **10**, 221.
[279] J. G. Buchanan, A. R. Edgar, and D. G. Large, *Chem. Comm.*, 1969, 558.
[280] A. M. Yurkevich, L. S. Varshavskaya, and I. I. Kolodkina, *Zhur. obshchei Khim.*, 1968, **38**, 2115.
[281] H. Shimanouchi, N. Saito, and Y. Sasada, *Bull. Chem. Soc. Japan.*, 1969, **42**, 1239.

7
Halogenated Sugars

Active interest has been shown in halogenated sugars, in particular in fluorinated compounds.

Glycosyl Halides

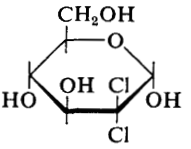
A new method for synthesising glycosyl fluorides under mild homogeneous conditions and in high yields involved treating the corresponding chlorides with silver tetrafluoroborate in ether or toluene.[282] The preparation of 2,3-unsaturated glycosyl fluorides from glycal esters is noted in Chapter 14.

Several further papers have appeared on the adducts formed on treating glycal derivatives with halogenating reagents. Continuing their studies of fluorinated carbohydrates, Hall and Manville have published the full details of their investigations into the products of addition of 'BrF' and 'IF' to glycals and 3,4-dihydro-2*H*-pyran derivatives. The adducts were characterised by n.m.r. methods and were found to have the fluorine at the anomeric positions, and, in the main, to have *trans* structures. Mechanisms of all the reactions were considered in detail.[283] A comparison was then made of the addition of 'ClF' to tri-*O*-acetyl-D-glucal with that of 'BrF' and 'IF'. Chlorine added to a suspension of tri-*O*-acetyl-D-glucal and silver fluoride in acetonitrile–benzene gave a mixture of the four possible glycosyl fluoride adducts with the *β-gluco*-compound dominant. The products were characterised by n.m.r. and their structures were confirmed by independent syntheses.[284] Crystallographic studies of the major product obtained with 'BrF' have shown it to be 3,4,6-tri-*O*-acetyl-2-bromo-2-deoxy-α-D-mannopyranosyl fluoride.[285] This result confirmed earlier n.m.r. findings. Results of detailed n.m.r. investigations of glycosyl fluorides are given in Chapter 23.

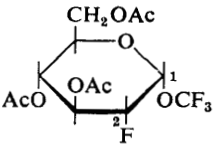
Chlorination of tri-*O*-acetyl-D-glucal in daylight has been reported to yield the α-*gluco*- and the *β-manno*-adducts in the ratio 4 : 1. Hydrolysis of the major product with hydrochloric acid yielded 2-chloro-2-deoxy-D-glucose, but under similar conditions the *manno*-isomer gave 2-deoxy-D-*arabino*-hexose. Dehydrochlorination of the glucosyl chloride with

[282] K. Igarashi, T. Honma, and J. Iriswa, *Carbohydrate Res.*, 1969, **11**, 577.
[283] L. D. Hall and J. F. Manville, *Canad. J. Chem.*, 1969, **47**, 361.
[284] L. D. Hall and J. F. Manville, *Canad. J. Chem.*, 1969, **47**, 379.
[285] J. C. Campbell, R. A. Dwek, P. W. Kent, and C. K. Prout, *Carbohydrate Res.*, 1969, **10**, 71.

trimethylamine afforded tri-*O*-acetyl-2-chloro-D-glucal, chlorination of which gave 3,4,6-tri-*O*-acetyl-2,2-dichloro-2-deoxy-D-*arabino*-hexosyl chloride, from which crystalline 2,2-dichloro-2-deoxy-D-*arabino*-hexose (79) was obtained by acidic hydrolysis (*cf.* vol. 2, p. 75).[286] In related work 2-deoxy-2-fluoro-D-glucose was prepared by the action of fluoro-oxytrifluoromethane on tri-*O*-acetyl-D-glucal. Four products (80)—(83) were formed in yields of 26, 34, 6, and 8% respectively; (80) and (81) could be converted to the required fluoro-sugar, making this procedure preferable to that described on p. 62. Structures of products were established by ^{1}H and ^{19}F n.m.r. spectroscopy.[287]



(79)



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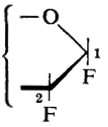
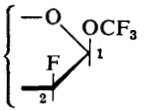
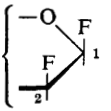


(81)

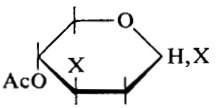


(82)

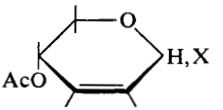


(83)

In agreement with results recently obtained with tri-*O*-acetyl-D-glucal, di-*O*-acetyl-D-xylal, on treatment with hydrogen chloride or hydrogen bromide in benzene, gave only small amounts of the adducts and mainly compounds (84, X = Cl or Br) plus small amounts of the *erythro*-isomers. The reaction occurred by addition to the unsaturated intermediate (85, X = Cl or Br). Di-*O*-acetyl-D-arabinal gave mainly the 2-deoxy-glycosyl halides because the C-4 acetoxy-group cannot assist so well the departure



(84)



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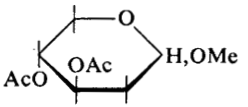

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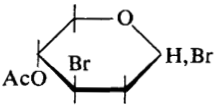


(87)

[286] J. Adamson and A. B. Foster, *Carbohydrate Res.*, 1969, **10**, 517.
[287] J. Adamson, A. B. Foster, L. D. Hall, and R. H. Hesse, *Chem. Comm.*, 1969, 309.

of the acetoxy-group from C-3, and thus unsaturated intermediates are formed. Di-*O*-benzoyl-D-arabinal and -xylal, on treatment with hydrogen chloride and bromide in benzene, underwent additions almost exclusively. Compound (86), when treated with hydrogen bromide, gave, in addition to the corresponding glycosyl bromide, some of the dibromo-derivative (87) *via* diacetyl-D-xylal.[288]

A most interesting effect governing the ease of solvolysis of glycosyl halides has been noted by Fletcher's group. The influence of *p*-nitrobenzoyl esters at positions other than C-2 on otherwise benzylated glycopyranosyl bromides was investigated, and marked decreases in solvolysis rates were noted as the ester groups were introduced. Compounds with C-2 benzyl substituents and either benzyl or *p*-nitrobenzoyl groups at positions 3 to 6 were synthesised from the corresponding 1-*O*-*p*-nitrobenzoyl esters by treatment with hydrogen bromide in dichloromethane. Irrespective of the configurations of the initial 1-esters, β-bromides were formed initially, and it was considered probable that they arose because of steric protection of the α-side of C-1 carbonium ions by the C-2 ether groups. Following their formation, the β-bromides anomerised at rates which decreased with the number of *p*-nitrobenzoyl esters present in the molecules. The β-bromides were more reactive than their anomers, but, regardless of the anomeric configuration, the main methanolysis products were α-glucosides. Anomerisations were therefore possible, and the products were formed by way of the more reactive β-bromides. 2-*O*-Benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide is readily accessible and can be converted to α-glycosides with high specificity; it was therefore recommended as being useful in α-glycoside synthesis. In the presence of tetrabutylammonium bromide, the corresponding α-bromide also gave the methyl α-glucoside in 95% yield.[64]

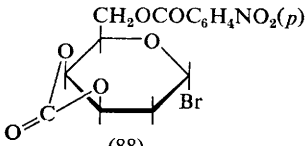
A study has been made of the acid and alkaline hydrolyses of α- and β-D-glucopyranosyl, α-D-galactopyranosyl, α-D-xylopyranosyl, and β-L-arabinopyranosyl fluorides. With the exception of the β-D-*gluco*-compound, all the acid reactions proceeded by *A*-1 mechanisms. The alkali-catalysed reactions were found to be of three types. The pentose derivatives gave free sugars, showed first-order kinetics, and appeared to react by nucleophilic hydroxide attack. The α-hexosyl compounds gave mixtures of free sugars and 1,6-anhydropyranoses, whereas the β-glucosyl fluoride gave predominantly the 1,6-anhydride, reacting 5000 times faster than the α-anomer, which suggested that the C-2 hydroxy-group participated in the fluoride displacement.[289]

The previously reported xylopyranosyl chloride 2,3,4-tri(chlorosulphates), produced by treatment of the free sugar with sulphuryl chloride, have been shown to be formed with inversion of configuration at C-1, presumably by way of the 1-chlorosulphates. Thus, α-D-xylopyranose gave the β-chloride,

[288] K. Bock, I. Lundt, and C. Pedersen, *Acta Chem. Scand.*, 1969, **23**, 2083.
[289] J. E. G. Barnett, *Carbohydrate Res.*, 1969, **9**, 21.

and a mixture of anomers gave a related mixture of glycosyl chlorides. The n.m.r. spectra of the anomeric chlorides and corresponding lyxo-pyranosyl compounds showed each to adopt the conformation in which the chlorine was axial. The *β-xylo*-compound therefore provided another example of a six-membered ring compound having all its ring substituents axial.[290]

A new crystalline glycosyl bromide (88) has been prepared by standard procedures and was used to prepare nucleosides from thymine, cytosine, and uracil,[291] and also cardenolides.[291a]



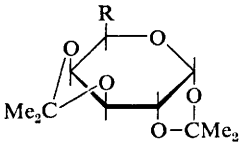
(88)

The anomeric configuration of 2-deoxy-3,5-di-*O*-*p*-toluyl-α-D-*erythro*-pentofuranosyl chloride has been assigned on the grounds of n.m.r. evidence,[292] and it has been shown that 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose is converted into the corresponding α-D-glucopyranosyl chloride more efficiently and with fewer side-reactions with an equimolar mixture of zinc chloride and thionyl chloride than with thionyl chloride alone.[293]

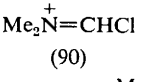
Other Halogenated Derivatives

Details of the reactions of (halogenomethylene)dimethyliminium halides with carbohydrate derivatives have now been reported in full (see vol. 1, p. 86), and mechanistic aspects have been discussed. Compound (89) with the chloride (90) in 1,1,2-trichloroethylene gave the intermediate (91) which, on treatment with aqueous bicarbonate, afforded mainly the formate ester, but on heating the reaction mixture, high yields of the chlorodeoxy-compound (92) were obtained. With (93), compound (89) gave an adduct which could readily be converted to the 6-acetate by hydrolysis. Compound (94) with (90) gave the formate ester when the reaction was carried out at room temperature; at elevated temperatures, however, the primary chloride (95) was formed after an acetal migration. At room temperature (96) gave (97) on treatment with (90), and at elevated temperatures, (98), again formed by acetal migration. In related fashion, (99) on heating with (90) gave (100), whilst (101) gave a complex mixture of products, one of which was the 2-ene.[294]

[290] H. J. Jennings, *Canad. J. Chem.*, 1969, **47**, 1157.
[291] W. W. Zorbach, S. L. De Bernardo, and K. V. Bhat, *Carbohydrate Res.*, 1969, **11**, 413.
[291a] W. W. Zorbach, S. L. De Bernardo, and K. V. Bhat, *Carbohydrate Res.*, 1969, **11**, 567.
[292] P. Nuhn, A. Zschunke, D. Heller, and G. Wagner, *Die Pharmazie*, 1969, **24**, 237.
[293] V. D. Grob, T. G. Squires, and J. R. Vercellotti, *Carbohydrate Res.*, 1969, **10**, 595.
[294] S. Hanessian and N. R. Plessas, *J. Org. Chem.*, 1969, **34**, 2163.



(89) R = CH_2OH
(91) R = $CH_2OCH{=}\overset{+}{N}Me_2$
(92) R = CH_2Cl



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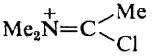

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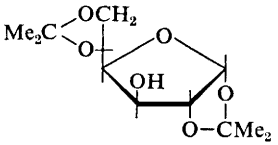
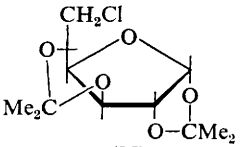


(94)



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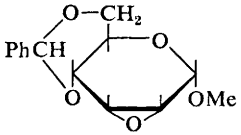

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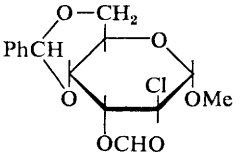
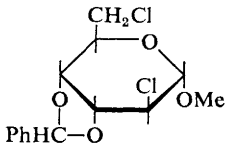
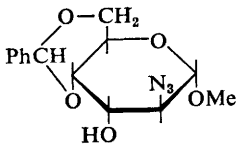


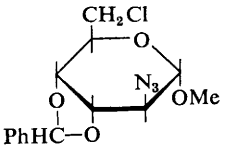
(97)



(98)



(99)



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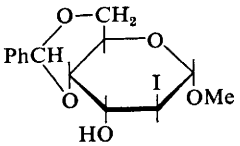


(101)

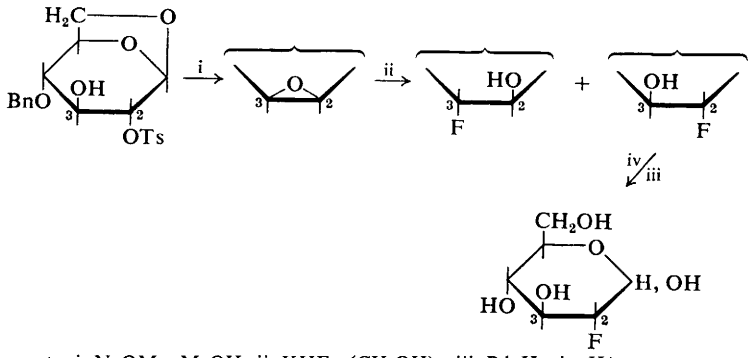
Chloro- and bromo-methylenedimethylammonium chloride have been found to be the halogenating agents formed when arsenic trichloride and tribromide are used in DMF for the halogenation of 2′,3′-protected ribofuranosyl nucleosides. With uridine itself, 5′-specificity was observed.[295]

Halogenated derivatives formed by additions to unsaturated carbohydrates are described in Chapter 14, and the *X*-ray-crystallographic analyses of some chlorodeoxy-compounds are noted in Chapter 24.

A review lecture on fluoro-sugars has been published; a useful survey of the methods used to synthesise these derivatives was made and brief comments on their biological activity were provided.[296]

A synthesis of 2-deoxy-2-fluoro-D-glucose has already been described [287] and a second has been reported, shown in Scheme 30. As was to

[295] R. F. Dods and J. S. Roth, *J. Org. Chem.*, 1969, **34**, 1627.
[296] P. W. Kent, *Chem. and Ind.*, 1969, 1128.



Reagents: i, NaOMe, MeOH; ii, KHF_2, $(CH_2OH)_2$; iii, Pd–H_2; iv, H^+

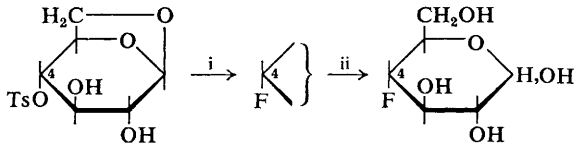
Scheme 30

be expected from diaxial epoxide ring opening, the *gluco*-isomer was the main product.[297]

More biological tests on 3-deoxy-3-fluoro-D-glucose have been reported. The originally described oxidation of the sugar by cell suspensions of *Saccharomyces cerevisiae* (see vol. 1, p. 85) has now been shown to be wrong. However, it has been confirmed that the sugar is incorporated into the cell and causes blockage of D-glucose and D-galactose metabolism.[298] Other workers have confirmed that the sugar is not metabolised by the yeast but showed that it stimulates respiration and endogenous metabolism. It is oxidised by D-glucose oxidase, but much more slowly than is D-glucose.[299] A variety of D-glucose derivatives modified at C-3 (including 3-deoxy-3-fluoro-D-glucose) and related compounds were tested for their active intestinal transport. The parent sugar and the 3-halogeno-derivatives were transported more effectively than was the 3-deoxy-compound, which indicated an attachment by hydrogen bonding between the carrier and the C-3 substituent. 3-Chloro- and 3-bromo-3-deoxy-D-glucose were prepared by treating 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose with lithium halides in DMF in the presence of calcium carbonate.[300]

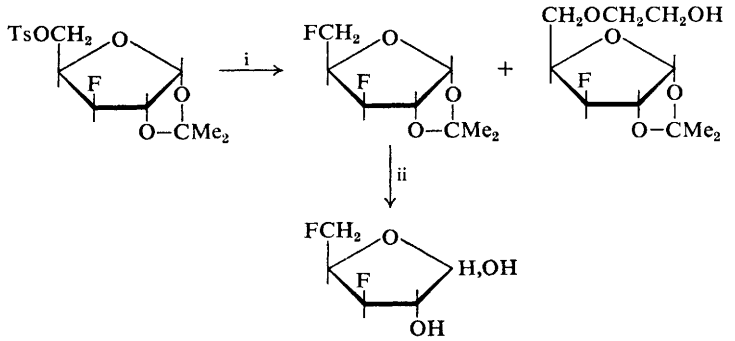
4-Deoxy-4-fluoro-D-glucose has been synthesised as shown in Scheme 31,[301] and the conversion of 3-deoxy-3-fluoro-5-*O*-toluene-*p*-sulphonyl-1,2-*O*-isopropylidene-α-D-xylofuranose into the first difluorosugar, namely 3,5-dideoxy-3,5-difluoro-D-xylose, has been reported. A by-product of the displacement was the 5-*O*-hydroxyethyl ether (Scheme 32).[302] 2-Deoxy-2-fluoro-D-arabinose (102) was prepared as shown in Scheme 33, and the

[297] J. Pacák, Z. Točík, and M. Černy, *Chem. Comm.*, 1969, 77.
[298] B. Woodward, N. F. Taylor, and R. V. Brunt, *Biochem. J.*, 1969, **114**, 445.
[299] R. J. Miles and S. J. Pirt, *Biochem. J.*, 1969, **114**, 10P.
[300] J. E. G. Barnett, A. Ralph, and K. A. Munday, *Biochem. J.*, 1969, **114**, 569.
[301] A. D. Barford, A. B. Foster, J. H. Westwood, and L. D. Hall, *Carbohydrate Res.*, 1969, **11**, 287.
[302] A. B. Foster and R. Hems, *Carbohydrate Res.*, 1969, **10**, 168.



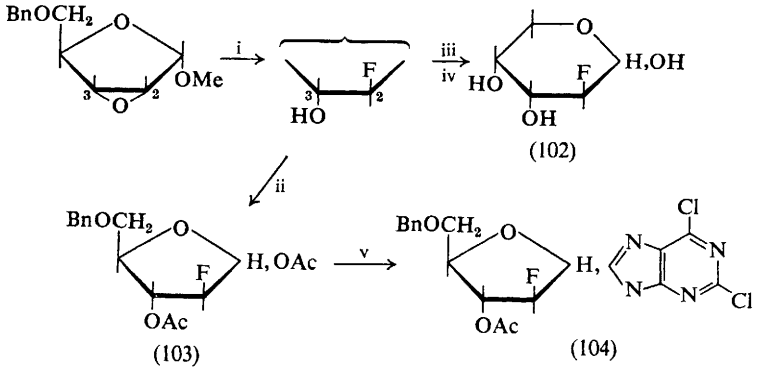
Reagents: i, KHF_2, $(CH_2OH)_2$; ii, H^+

Scheme 31



Reagents: i, KHF_2, $(CH_2OH)_2$; ii, H^+

Scheme 32



Reagents: i, KHF_2, $(CH_2OH)_2$; ii, acetolysis; iii, H_2–Pd; iv, H^+; v, 2,6-dichloropurine

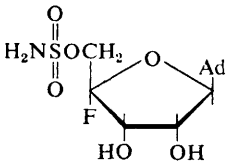
Scheme 33

derived furanosyl acetate (103), on fusion with 2,6-dichloropurine, gave the purine derivative (104).[303]

The novel structure (105) has been assigned to nucleocidin, largely on the basis of 100 MHz ^1H and ^{19}F n.m.r. and mass spectrometric evidence. This nucleoside antibiotic, therefore, has the remarkable feature of having

[303] J. A. Wright, N. F. Taylor, and J. J. Fox, *J. Org. Chem.*, 1969, **34**, 2632.

fluorine attached to the sugar ring and is thus the first known, naturally occurring fluorinated sugar derivative.[304]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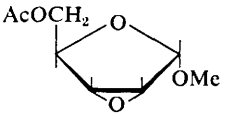
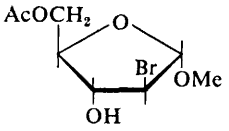
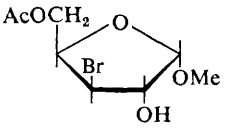

(106)


(107)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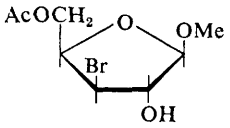
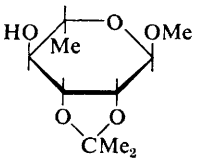
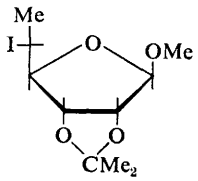
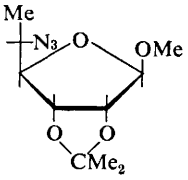
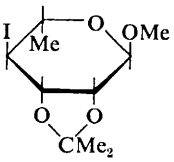

(109)

Bromodeoxybenzoates, formed on treatment of benzylidene acetals with *N*-bromosuccinimide in refluxing carbon tetrachloride, have been examined in detail.[194–196] Related compounds have also been prepared from epoxides. Reaction of anhydrous magnesium bromide with the α-D-ribofuranoside (106) gave a mixture of (107) and (108), whereas from the β-anomer only (109) was formed. The reactions were compared with previously obtained results on the ring-opening of the anomeric methyl 5-*O*-acetyl-2,3-anhydro-D-lyxofuranosides. It was concluded that the methoxy-groups exhibited a


(110)


(111)


(112)


(113)

stronger steric effect than the acetoxymethyl groups; possible reasons for this were discussed in terms of the conformations of the bicyclic ring systems.[305]

[304] G. O. Morton, J. E. Lancaster, G. E. Van Lear, W. Fulmor, and W. E. Meyer, *J. Amer. Chem. Soc.*, 1969, **91**, 1535.

[305] E. J. Reist and S. L. Holton, *Carbohydrate Res.*, 1969, **9**, 71.

A repetition of the reaction of triphenyl phosphite methiodide with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (110) gave mainly (111) by a ring-contraction process similar to that which has been observed to occur during nucleophilic displacement reactions carried out on the methanesulphonate of (110). After azidolysis of the mother liquors of the reaction, (112) and (113) were isolated.[306] The synthesis of further azido-sugars from deoxyhalogeno-compounds is mentioned in Chapter 10.

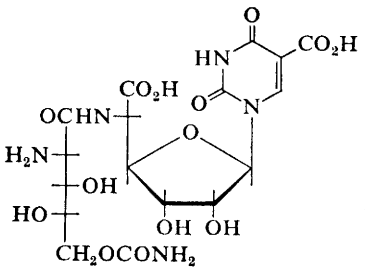
[306] K. Kefurt, J. Jarý, and Z. Samek, *Chem. Comm.*, 1969, 213.

8
Amino-sugars*

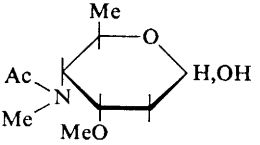
Volume IA of a comprehensive survey of the chemistry and biochemistry of amino-sugars has appeared;[307] it contains chapters on monosaccharide amino-sugars (Horton), sialic and muramic acids (Blix and Jeanloz), oligosaccharides (Baer), and nucleotide derivatives (Strominger). Unfortunately, there are few references to work published after 1966.

Natural Products†

A family of peptide nucleosides, the polyoxins A—L, have been described in which the sugar moiety is 5-amino-5-deoxy-D-alluronic acid, itself an α-amino-acid. The substituent at C-5 of the uracil moiety varied widely. The simplest member of the series was (114).[308] D-Rhodosamine, a component of megalomicins A, B, and C, has been shown to be 2,3,6-trideoxy-3-dimethylamino-D-*lyxo*-hexopyranose.[309] The structure of the carbohydrate component of the glyco-steroid holacurtin, namely 2,4,6-trideoxy-4-(*N*-methyl)acetamido-3-*O*-methyl-D-*ribo*-hexose (115), has been confirmed by synthesis.[310]



(114)



(115)

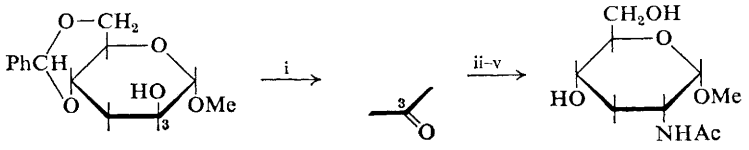
[307] 'The Amino-Sugars,' vol. IA, ed. R. W. Jeanloz, Academic Press, New York, 1969.
[308] K. Isono, K. Asahi, and S. Sukuki, *J. Amer. Chem. Soc.*, 1969, **91**, 7490.
[309] A. K. Mallams, *J. Amer. Chem. Soc.*, 1969, **91**, 7505.
[310] J. Hildesheim, S. D. Géro, G. Khuong-Huu, and C. Monneret, *Tetrahedron Letters*, 1969, 2849.

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

The methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside structure has been confirmed for methyl perosaminide, by an n.m.r. study on its peracetate.[311]

Synthesis*

Many new synthetic amino-sugars have been described during the past year. Several syntheses have used oximes as intermediates. Methyl 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranoside (116) has been prepared as shown in Scheme 34 [312] (see also p. 151). Methyl 3-acetamido-4,6-

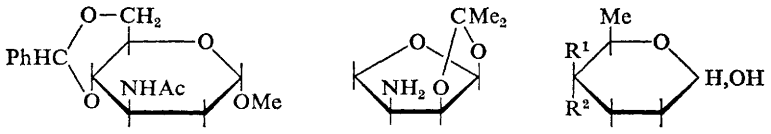


(116)

Reagents: i, DMSO, Ac_2O; ii, NH_2OH, HCl; iii, LAH; iv, Ac_2O; v, H^+

Scheme 34

O-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (117) was prepared in a similar manner, but in this case, reduction of the oxime precursor gave *arabino* and *ribo* products in the ratio 3 : 1.[312] In another report LAH reduction of the appropriate *syn*-oxime gave the axial amino-product, as did catalytic hydrogenation.[313] Reduction of the oxime of 1,2-*O*-isopropylidene-α-L-*glycero*-tetro-3-ulofuranose with LAH gave the amino-sugar (118).[314] Oxime intermediates were also used in the synthesis of forosamine (119) and its D-*threo* isomer (120).[315]



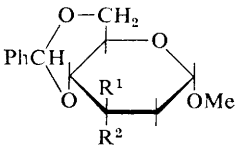
(117) (118) (119) $R^1 = H$, $R^2 = NMe_2$
(120) $R^1 = NMe_2$, $R^2 = H$

Sulphonate esters have also been used either directly or indirectly in the synthesis of amino-sugars. *N*-Acetyl-lincosamine (6-acetamido-6,8-dideoxy-D-*erythro*-D-*galacto*-octose) has been prepared (see p. 152). Compound

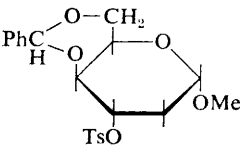
[311] C. H. Lee and C. P. Schaffner, *Tetrahedron*, 1969, **25**, 2229.
[312] A. Rosenthal and P. Catsoulacos, *Canad. J. Chem.*, 1969, **47**, 2747.
[313] P. J. Benyon, P. M. Collins, and W. G. Overend, *J. Chem. Soc.* (*C*)., 1969, 272.
[314] J. M. J. Tronchet, R. Graf, and J. Tronchet, *Helv. Chim. Acta*, 1969, **52**, 315.
[315] E. L. Albano and D. Horton, *Carbohydrate Res.*, 1969, **11**, 485.

* See also Chapters 6 and 20.

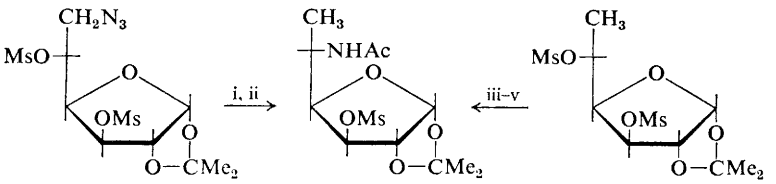
(121) has been synthesised *via* displacement of the epimeric sulphonyloxy-compound with azide ion,[313] as has its epimer (122). Similar displacements when attempted in the *cis*-fused ring analogue (123) gave the 2,3-unsaturated



(121) $R^1 = H$, $R^2 = NH_2$
(122) $R^1 = NH_2$, $R^2 = H$



(123)

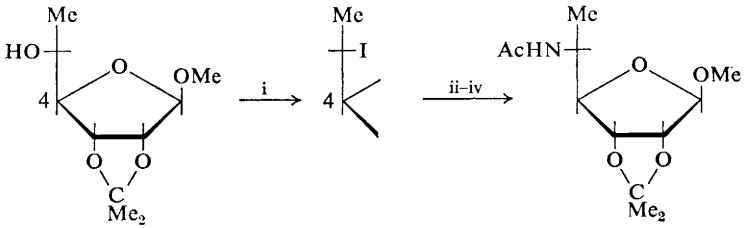


(124)

Reagents: i, Ni, NH_2NH_2; ii, Ac_2O; iii, NaN_3; iv, reduction; v, Ac_2O

Scheme 35

product. 5-Acetamido-5,6-dideoxy-1,2-*O*-isopropylidene-L-idofuranose (124) has been prepared by the methods shown in Scheme 35; one involved direct displacement, the other transfer of nitrogen from C-6 to C-5 *via* an epimine intermediate.[316] A similar displacement reaction has been used in the synthesis of methyl 5-acetamido-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-allofuranoside (Scheme 36).[317] The preparation of the 3-amino-3-deoxy- and 6-amino-6-deoxy-derivatives (as their hydrochlorides) of both D-glucose and D-allose has been described.[318] Full details have appeared



Reagents: i, $(PhO)_3P$,MeI; ii, NaN_3; iii, LAH; iv, Ac_2O

Scheme 36

[316] C. F. Gibbs and L. Hough, *Chem. Comm.*, 1969, 1210.
[317] A. I. Usov, K. S. Adamyants, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 697.
[318] J. Jarý, Z. Kefurtová, and J. Kovář, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1452.

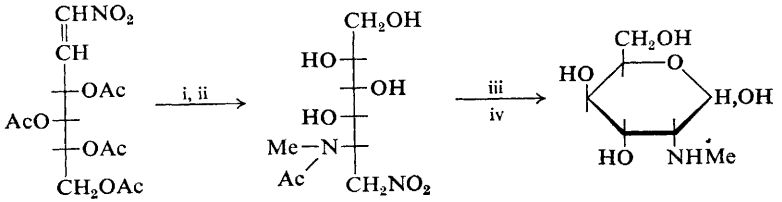
(see vol. 2, p. 65) of the synthesis of 4-amino-4,6-dideoxy-L-mannose (L-perosamine).[318a]

The synthesis of 6-amino-6-deoxy-D-glucose-6-^{15}N has been described.[319] Reaction of sodium azide in HMPT with 1,2-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-xylofuranose gave 3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose and thence 3-amino-3-deoxy-D-ribose.[320]

The synthesis of methyl 4-amino-4-deoxy-D-glucosiduronic acid has been achieved;[321] this compound is related to the carbohydrate moiety of gougerotin.

Amino-sugars have also been prepared by modification of molecules already containing this functional group. A new synthesis of 3-acetamido-2,3,6-trideoxy-D-*lyxo*-hexose (*N*-acetyl-D-daunosamine), as well as one of its D-*arabino*-isomer, has been described, starting from the corresponding 6-hydroxy-derivatives.[322] Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranoside has been prepared from the corresponding *gluco* isomer.[323]

Nitro-sugars have been used by several groups as intermediates. Perry's group have obtained several 2-acetamido-2-deoxy-hexoses[324] and heptoses[325, 326] by orthodox procedures from per-*O*-acetyl-1-nitro-hex-1-enes and -hept-1-enes. The same type of sequence has been used in the synthesis of 2-deoxy-2-methylamino-D-gulose (Scheme 37.)[327] The dialdehyde

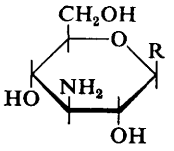


Reagents: i, $MeNH_2$, MeOH; ii, Ac_2O; iii, NaOH; iv, HCl

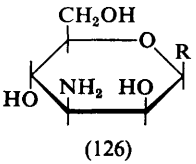
Scheme 37

obtained by periodate oxidation of 6-dimethylamino-9-(β-D-ribofuranosyl)-purine has been used in the nitromethane cyclisation reaction to give a mixture of products that were separated and reduced to the derivatives (125)—(127).[328] Similar reaction on the dialdehyde from inosine gave the

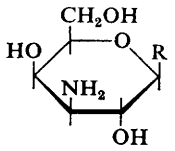
[318a] J. S. Brimacombe, O. A. Ching, and M. Stacey, *J. Chem. Soc.* (*C*), 1969, 1270.
[319] B. Coxon, *Carbohydrate Res.*, 1969, **11**, 153.
[320] J. Defaye and A. M. Miquel, *Carbohydrate Res.*, 1969, **9**, 250.
[321] M. P. Kotick, R. S. Klein, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1969, **11**, 369.
[322] H. H. Baer, K. Čapek, and M. C. Cook, *Canad. J. Chem.*, 1969, **47**, 89.
[323] K. Miyai, H. K. Zimmerman, and P. H. Gross, *J. Org. Chem.*, 1969, **34**, 1635.
[324] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1969, **47**, 1245.
[325] C. F. Gibbs, D. T. Williams, and M. B. Perry, *Canad. J. Chem.*, 1969, **47**, 1479.
[326] D. T. Williams and M. B. Perry, *Canad. J. Chem.*, 1969, **47**, 4493.
[327] Y. Ito, Y. Ohashi, and T. Miyagishima, *Carbohydrate Res.*, 1969, **9**, 125.
[328] F. W. Lichtenthaler and H. P. Albrecht, *Chem. Ber.*, 1969, **102**, 964.



(125)

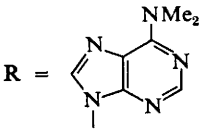


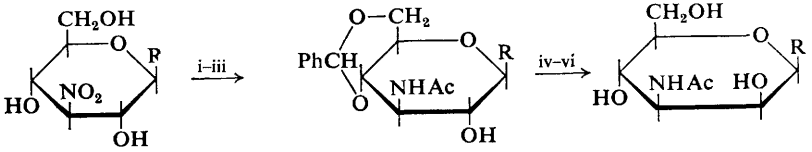
(126)



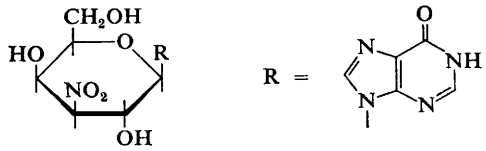
(127)

gluco and *galacto* compounds (128) and (129). Both products were inverted at C-2 by the method shown in Scheme 38 for (128).[329] 5′-Amino-5′-deoxy-derivatives of adenosine and guanosine have been described.[330]





(128)



(129)

Reagents: i, Ni–H_2; ii, Ac_2O, MeOH; iii, PhCHO, $ZnCl_2$; iv, MsCl, pyr; v, NaOAc, H_2O, $MeOCH_2CH_2OH$; vi, H^+

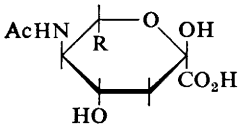
Scheme 38

An improved synthesis of *N*-acetylneuraminic acid has been described. Condensation of 2-acetamido-2-deoxy-D-mannose with pyruvate or oxalate gave four isomeric products from which the required one could be isolated in about 10% yield, but this was doubled by the addition of borate ions. A major factor is that borate ions reduce the degree of alkaline epimerisation of 2-acylamino-2-deoxy-aldoses.[331] The *N*-[^{14}C]-acetyl derivative of

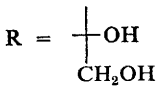
[329] F. W. Lichtenthaler, P. Emig, and D. Bommer, *Chem. Ber.*, 1969, **102**, 971.
[330] M. G. Stout, M. J. Robins, R. K. Olsen, and R. K. Robins, *J. Medicin. Chem.*, 1969, **12**, 658.
[331] M. J. How, M. D. A. Halford, and M. Stacey, *Carbohydrate Res.*, 1969, **11**, 313.

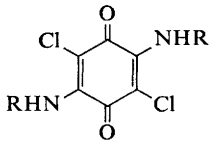
neuraminic acid has been prepared by use of [^{14}C]-acetic anhydride with the methyl β-glycoside followed by a mild acid hydrolysis.[332] The synthesis of 5-acetamido-3,5-dideoxy-D-*galacto*-octulosonic acid (130), an eight-carbon analogue of *N*-acetylneuraminic acid, has been reported.[333] Unsaturated compounds related to neuraminic acid have been described (see p. 113).

The 6-amino-6-deoxy-D-galactose derivative (131) has been prepared,[334] as has the amino-amido-derivative (132).[335] Several steroid glycosides of



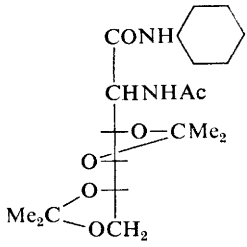
(130)





R = 1,2:3,4-di-*O*-isopropylidene-D-galactopyranosyl

(131)



(132)

2-acetamido-2-deoxy-β-D-glucopyranose have been reported.[96] The synthesis of glycosides of amino-sugars is discussed in Chapter 3. The synthesis of 6-amino-heptose derivatives has been described (see p. 91) and a series of papers has appeared on the preparation of racemic 3-amino-sugars (see p. 117).

A route to amino-sugars bearing a branch at the C–NH_2 carbon atom has been described (see p. 88).

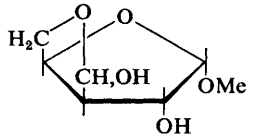
Papers on the synthesis of unsaturated derivatives of amino-sugars are described on pages 112—114.

Reactions

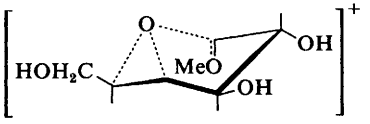
Nitrous acid deamination of methyl 4-amino-4-deoxy-α-D-glucopyranoside [*cf.* solvolysis of the corresponding 4-*p*-nitrobenzenesulphonate (vol. 1, p. 81), and p. 57] gave six products, including methyl α-D-glucopyranoside (35%), methyl β-L-altrofuranoside (7%), glucose, and the ring-contracted product (133) (15%).[336] Glucose was shown not to be a primary product, but to arise during the work-up. It was postulated as formed from 4,5-anhydro-D-galactose, a small amount of which was isolated and shown

[332] G. V. Wirtz-Peitz, R. Schauer, and H. Faillard, *Z. physiol. Chem.*, 1969, **350**, 111.
[333] R. McLean and J. Beidler, *J. Amer. Chem. Soc.*, 1969, **91**, 5388.
[334] V. I. Veksler and Z. Ya. Khavin, *Zhur. obshchei. Khim.*, 1968, **38**, 2122.
[335] M. F. Shostakovskii, K. F. Lavrova, N. N. Aseeva, and A. I. Polyakov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1168.
[336] N. M. K. Ng Ying Kin, J. M. Williams, and A. Horsington, *Chem. Comm.*, 1969, 971.

to be extremely unstable. The various products were thought to be formed *via* the ion (134).

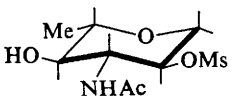


(133)

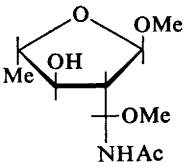


(134)

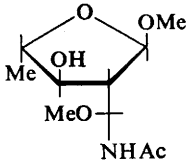
Reaction of methyl 3-acetamido-3,6-dideoxy-2-*O*-methanesulphonyl-α-L-glucopyranoside (135) with hot methanolic sodium methoxide gave two ring-contracted products, the structures of which were shown to be (136) and (137) by detailed n.m.r. studies.[337] In (135), the C-2-sulphonyloxy-bond is antiparallel with the C-3, C-4 bond, which favours ring-contraction. Reaction of the resulting carbonium ion with methoxide ion gives the epimeric products.



(135)



(136)

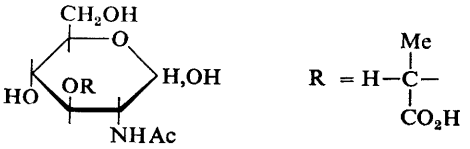


(137)

A possible micro-method for the specific detection of 2-acetamido-2-deoxy-β-glycosyl linkages in oligosaccharides has been proposed.[338] The method was based on the observation that acetylated 2-acetamido-2-deoxy-β-D-glucopyranosides underwent specific transglycosidation when heated with benzyl alcohol in the presence of zinc chloride. The procedure involved heating methylated derivatives in butyl acetate in the presence of butyl alcohol and zinc chloride. β-Glycosidic linkages were specifically cleaved as a consequence of participation of the C-2-acetamido-groups. The butyl glycosides produced can be examined by g.l.c. or t.l.c.

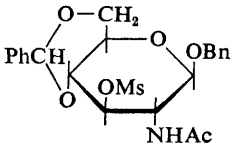
N-Acetylmuramic acid has been prepared by direct acetylation of the free amino-sugar or from the known benzamido-derivative by debenzoylation, acetylation, and removal of the blocking groups. The synthesis of the isomeric structure (138) was also described.[339] 4-*O*- and 6-*O*-acetyl, and 4,6-di-*O*-acetyl derivatives of *N*-acetylmuramic acid have been reported.[340] 2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-galactose (galactomuramic acid) and 2-amino-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-galactose (galacto-isomuramic acid) have been synthesised.[341]

[337] K. Čapek, J. Jarý, and Z. Samek, *Chem. Comm.*, 1969, 1162.
[338] W. L. Salo and H. G. Fletcher jun., *J. Org. Chem.*, 1969, **34**, 3026.
[339] R. Gigg and C. D. Warren, *J. Chem. Soc.* (*C*), 1969, 295.
[340] T. Osawa, P. Sinaÿ, M. D. A. Halford, and R. W. Jeanloz, *Biochemistry*, 1969, **8**, 3369.
[341] P. Sinaÿ and R. W. Jeanloz, *Carbohydrate Res.*, 1969, **10**, 189.

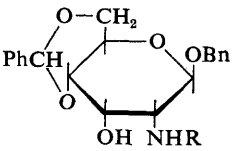


(138)

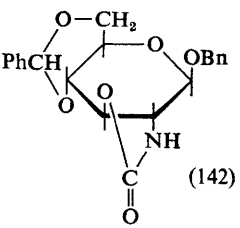
2-Amino-2-deoxy-D-allose derivatives have been prepared from *gluco* precursors, *e.g.* (139) to (140). However, the α-anomer of (139) was unreactive[323] (*cf.* p. 92). The *N*-benzoyl analogue of (139) gave the *allo* compound but also the 2,3-oxazoline. Compound (140) was hydrolysed to the unblocked compound (141) which was converted into the oxazolidone. In a further paper[342] the same group studied the chemistry of oxazolidenes, formed from both *cis*- and *trans*-amino-alcohol systems. Compound (142)



(139)



(140) R = Ac
(141) R = H



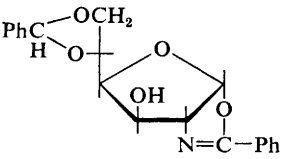
(142)

was obtained most successfully using *NN'*-carbonyldi-imidazole. The synthesis of the oxazoline (143) from 2-benzamido-2-deoxy-D-glucose has been described.[343]

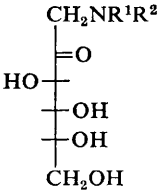
3-Amino-3-deoxy-D-galactopyranosyl nucleosides have been described.[344] The Maillard reaction has been applied to the synthesis of *N*- and *N'*-(1-deoxy-D-fructosyl)-L-lysine and their *N*-formyl derivatives. Related compounds of lactulose and lysine were also reported.[345]

The degradation of 1-amino-1-deoxy-2-fructoses (144) to 5-hydroxymethyl-2-furfuraldehyde (145) in acidic media has been examined.[346] When carried out in deuterium oxide the reaction afforded a product with varying amounts of deuterium incorporated in the aldehydic group and at the adjacent ring position. It was concluded that incorporation of the label in the aldehydic group was consistent with enolisation at positions 1 and 2, and that incorporation at position 3 followed 2,3-enolisation. However, under certain conditions, no detectable isotope incorporation occurred, so that (146) was not a necessary intermediate in the degradation process, as has been proposed.

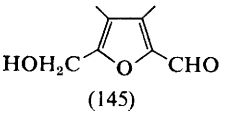
[342] K. Miyai and P. H. Gross, *J. Org. Chem.*, 1969, **34**, 1638.
[343] M. L. Shul'man, I. M. Privalova, and Ya. A. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2655.
[344] F. W. Lichtenthaler, G. Bambach, and P. Emig, *Chem. Ber.*, 1969, **102**, 994.
[345] P. A. Finot and J. Mauron, *Helv. Chim. Acta*, 1969, **52**, 1488.
[346] M. S. Feather and K. R. Russell, *J. Org. Chem.*, 1969, **34**, 2650.



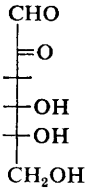
(143)



(144)



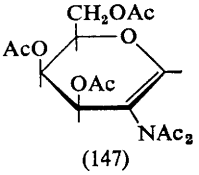
(145)



(146)

2-Amino-2-deoxy-DL-glyceraldehyde has been studied and shown to be stable only in acid solution; however, it gave normal aldehyde derivatives. The *N*-acetyl derivative, which has been identified only in solution and which is a possible product from the Smith degradation of amino-polysaccharides, was best isolated and characterised as its ethylenedithioacetal.[347]

Further studies on the reaction of isopropenyl acetate with acetamido-sugars have been described.[348] Acetylation of 1,3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose with the reagent caused smooth *N*-acetylation. The β-anomer, however, gave (147) in high yield (see also p. 112).



(147)

The addition of phosphonium salts to *aldehydo* derivatives of acetamido-sugars (*cf.* vol. 1, p. 134) has led to the synthesis of 4-amino-octose derivatives.[349] An extract from *Tetrahymena pyriformis* catalysed the transfer of a glucose residue to 2-amino-2-deoxy-D-glucose and gave 2-amino-2-deoxy-6-*O*-(α-D-glucopyranosyl)-D-glucose.[350]

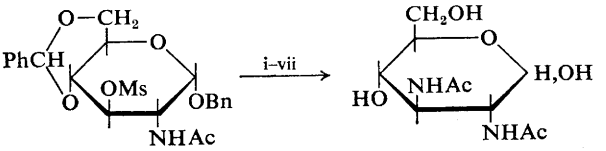
[347] S. David and A. Veyrieres, *Carbohydrate Res.*, 1969, **10**, 35.
[348] N. Pravdic and H. G. Fletcher jun., *Croat. Chem. Acta*, 1969, **41**, 125.
[349] M. N. Mirzayanova, L. P. Davydova, and G. I. Samokhvalov, *Zhur. obshehei Khim.*, 1968, **38**, 1954.
[350] A. L. Tarentino and F. Maley, *Arch. Biochem. Biophys.*, 1969, **130**, 80.

Physical Properties

It has been demonstrated that the α- and β-anomers of 2-amino-2-deoxy-D-glucopyranose have different dissociation constants for the amino-group, the α-anomer being the stronger base. Factors causing this difference were discussed and it was concluded that solvation differences were responsible.[351] A general study on the dissociation constants of several methyl amino-deoxy-4,6-*O*-benzylidene-hexosides has been reported [352] (*cf.* vol. 2, p. 92). The mass spectra of several 4,6-*O*-benzylidene-derivatives of 2-acetamido-2-deoxy-sugars have been recorded.[353] O.r.d., c.d., and n.m.r. studies are reported on pp. 183 and 174 respectively.

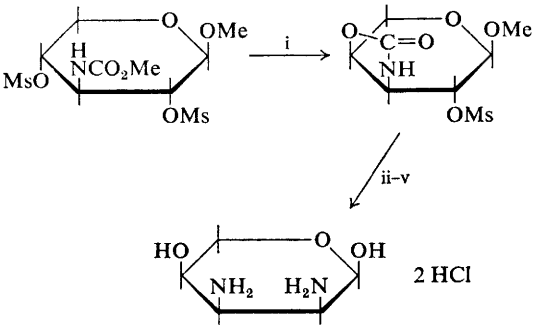
Diamino- and Polyamino-sugars

2,3-Diacetamido-2,3-dideoxy-D-glucose has been synthesised as shown in Scheme 39,[354] and 2,3-diamino-2,3-dideoxy-L-ribose has been prepared as shown by the sequence given in Scheme 40. No evidence was obtained for any intramolecular displacement of the methanesulphonyloxy-group



Reagents: i, NaOAc, H_2O, $MeOCH_2CH_2OH$; ii, MsCl, pyr; iii, NaN_3, DMF; iv, aq AcOH; v, H_2–Pd; vi, Ac_2O; vii, H_2–Pd

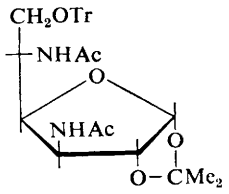
Scheme 39



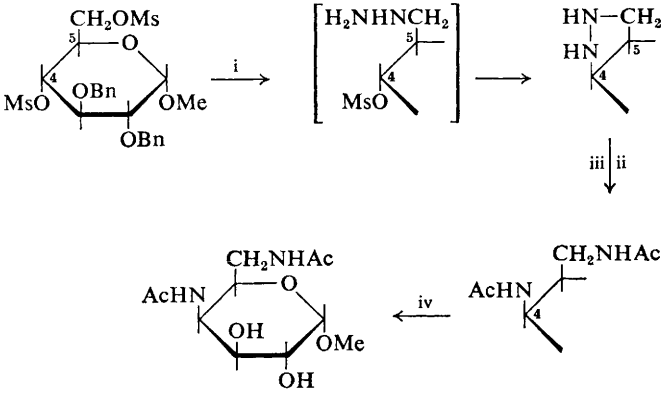
Reagents: i, NaF, DMF; ii, NaN_3, DMF; iii, Pd–H_2; iv, $Ba(OH)_2$; v, HCl

Scheme 40

[351] A. Neuberger and A. P. Fletcher, *J. Chem. Soc.* (*B*), 1969, 178.
[352] C. B. Barlow, R. D. Guthrie, and A. M. Prior, *Carbohydrate Res.*, 1969, **10**, 481.
[353] A. M. Stephen and D. C. De Bruyn, *S. Afr. Med. J.*, 1968, **42**, 794.
[354] W. Meyer zu Reckendorf, *Chem. Ber.*, 1969, **102**, 4207.

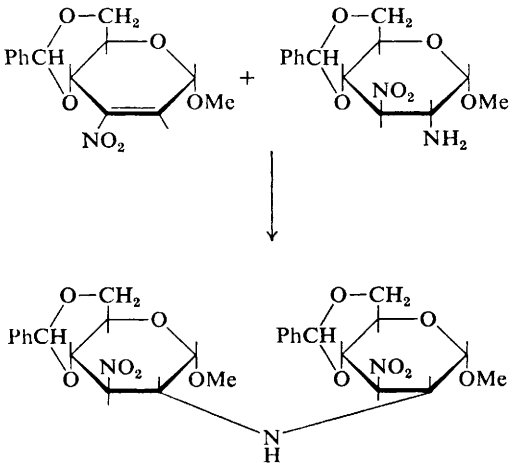


(148)



Reagents: i, NH_2NH_2; ii, NH_2NH_2–Ni; iii, Ac_2O, pyr; iv, H_2–Pd

Scheme 41



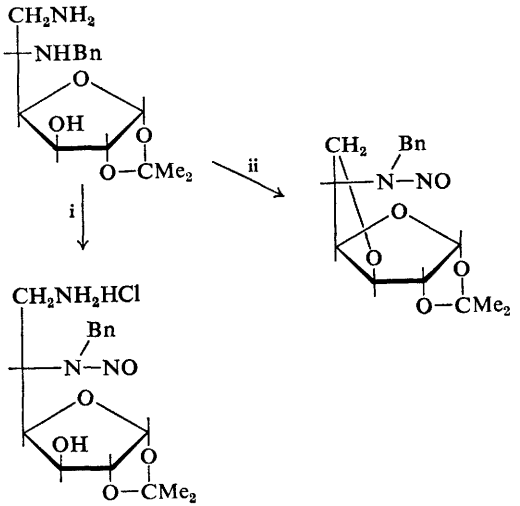
(149)

Scheme 42

at C-2.[355] A route to 2,3-diamino-2,3-dideoxy-D-galactose derivatives has been described (see p. 88).

Neighbouring group reactions giving pyrazolidine sugar derivatives have been examined as a route for the synthesis of diamino-sugars; for example Scheme 41. Compound (148) was prepared by a similar route.[356] 3,6-Diamino-3,6-dideoxy-D-glucose has been prepared *via* 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, by orthodox sequence.[357]

Novel types of diamino-sugar derivatives, for example (149), have been described by the route shown in Scheme 42.[358] Treatment of primary, secondary diamino compounds with nitrous acid has been examined; under weak acid conditions the secondary group was nitrosated whilst the primary one was protected by salt formation, whereas in strong acid, concurrent nitrosation and deamination occurred; see, for example, Scheme 43.[359]



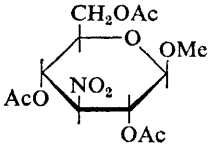
Reagents: i, HNO_2, HCl; ii, HNO_2

Scheme 43

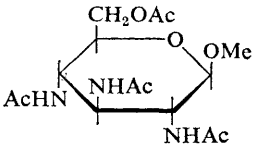
N-Acetyl triamino-sugars have been prepared by reaction of 3-deoxy-3-nitro-glycosides with ammonia, followed by standard steps. Thus (150) gave (151).[360]

[355] E. J. Reist and S. H. Cruse, *J. Org. Chem.*, 1969, **34**, 3029.
[356] H. Paulsen and D. Stoye, *Chem. Ber.*, 1969, **102**, 3833.
[357] J. Kovář and J. Jarý, *Coll. Czech. Chem. Comm.*, 1969, **34**, 2619.
[358] H. H. Baer and F. Rajabalee, *Canad. J. Chem.*, 1969, **47**, 4086.
[359] H. Paulsen and E. Mäckel, *Chem. Ber.*, 1969, **102**, 3844.
[360] F. W. Lichtenthaler, P. Voss, and N. Majer, *Angew. Chem. Internat. Edn.*, 1969, **8**, 211.

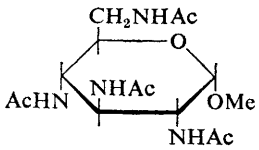
Attempts to synthesise 2,3,4,6-tetramino-2,3,4,6-tetradeoxy-D-glucose derivatives have been described.[268] The one successful, but tedious, route to methyl 2,3,4,6-tetra-acetamido-2,3,4,6-tetradeoxy-α-D-glucoside (152) proceeded *via* (153) which was subjected to standard reactions. A second route was proposed *via* the diazido-benzamido-derivative (154), and although this was successfully prepared, attempted oxidation with DMSO and acetic or benzoic anhydride gave only the ester in good yield. Since the C-3-OH could not be oxidised in (154), the C-3 epimer (155) was sought



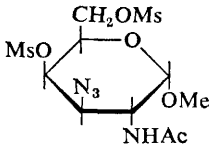
(150)



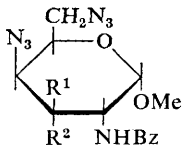
(151)



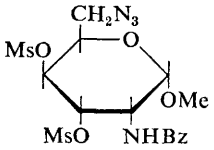
(152)



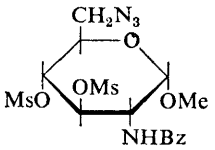
(153)



(154) $R^1 = OH$, $R^2 = H$
(155) $R^1 = H$, $R^2 = OH$



(156)

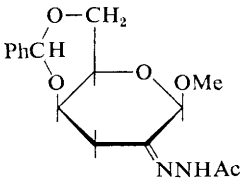


(157)

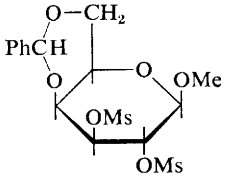
by another route in the hope that it could be successfully oxidised. Compound (155) was obtained (see p. 56), but it too gave only the C-3-ester in excellent yield when treated with DMSO and acetic or benzoic anhydride. A final attempt was proposed *via* (156) which was to be prepared from (157) *via* treatment with sodium benzoate in HMPT. However, only the C-3-sulphonyloxy-group could be displaced, the C-4-group being resistant to displacement even by azide ion in HMPT.[268]

9
Hydrazones, Osazones, and Formazans

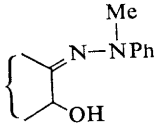
Continuing their investigations of reactions of carbohydrate derivatives with hydrazine, Paulsen and his colleagues [361] have examined the reaction of hydrazine with sugar hydrazones. The results and possible mechanistic pathways are illustrated (Scheme 44). The starting materials were prepared by hydrazine treatment of sulphonates; for example, (158) was prepared in low yield from the galactose derivative (159) (see also ref. 270).



(158)



(159)

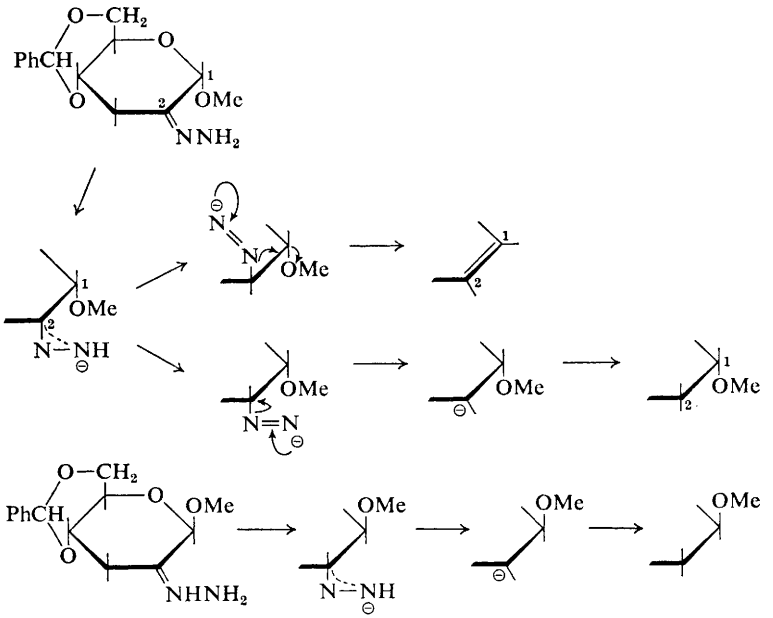


(160)

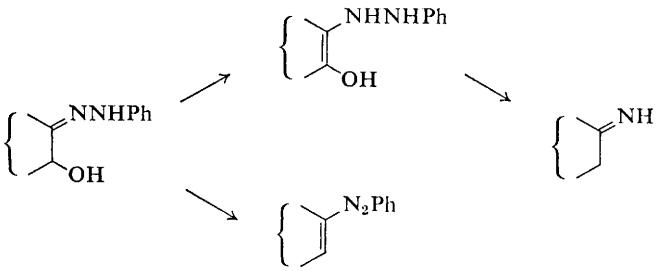
Work continues on the mechanism of osazone formation.[362] The rates of osazone formation from 2-hydroxy-cyclohexanone derivatives were measured and intermediates were identified. It was concluded that the initial hydrazone rearranged initially by two routes (Scheme 45). The *N*-methyl derivative (160) reacted faster than the parent compound, corresponding to a more rapid elimination of *N*-methylaniline. This result was in agreement with results with glucose phenylosazones and glucose *N*-methylphenylosazones.

The question of why sugar alkylphenylhydrazones form polyhydrazones, whereas sugar phenylhydrazones give osazones has been rediscussed.[363] It was suggested that the Fiesers' explanation, which invoked chelation

[361] H. Paulsen and D. Stoye, *Chem. Ber.*, 1969, **102**, 3824.
[362] H. Simon and W. Moldenhauer, *Chem. Ber.*, 1969, **102**, 1191.
[363] H. Simon, W. Moldenhauer, and A. Kraus, *Chem. Ber.*, 1969, **102**, 2777.



Scheme 44



Scheme 45

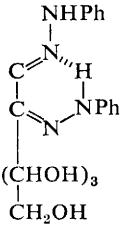
effects to account for the difference, was inadequate, and instead β-elimination from C-2—C-3 may play a role in preventing further reaction of phenylhydrazones. The results of experiments with trioses and tetroses as models were reported.

The reaction of D-glucose benzoylhydrazone with base has been investigated using ^{14}C-labelled materials.[364] The results indicated that for the formation of glyoxal bis(benzoylhydrazone), a labelled entity from C-1 and

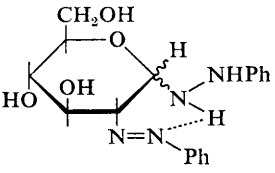
[364] C. S. Russell and R. Lyons, *Carbohydrate Res.*, 1969, **9**, 347.

C-2 of D-glucose benzoylhydrazone, competes with glycolaldehyde fragments from the non-labelled C-3—C-6 portion of the parent molecule for reaction with the benzoylhydrazone groups.

The evidence for the accepted structure of sugar osazones, *e.g.* (161), has been reinterpreted[365] and, in conjunction with evidence provided by i.r. and u.v. spectral data, has resulted in the proposal that in the solid state osazones have structures such as (162). In solution, structure (162) may be in equilibrium with the open-chain form.



(161)



(162)

The acyclic–cyclic relationship of aldose phenylhydrazones has been surveyed and an n.m.r. investigation of the *cis-trans*-isomerism about the double bonds in the acyclic species has been reported.[366]

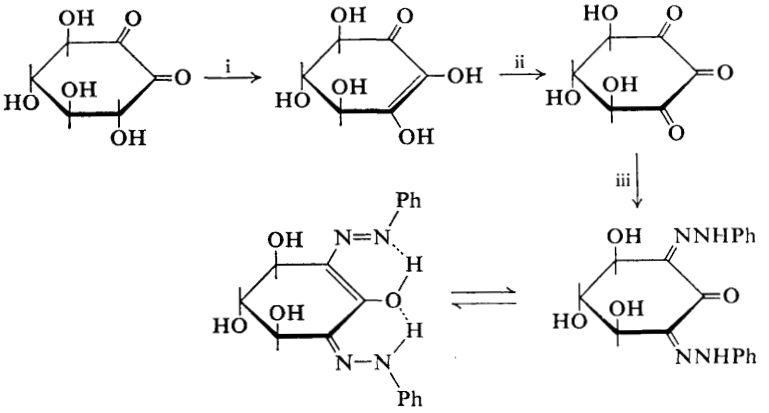
The mass spectra of the phenylosazones of several monosaccharides have been determined.[367]

DL-*xylo*-2-Oxo-1,3-bis(phenylhydrazono)-cyclohexane-4,5,6-triol (163) has been isolated from prolonged reactions between mono- or di-keto inositols and phenylhydrazine.[368] Compound (163) was the common product from D- and L-*myo*-inosose-1, *myo*-inosose-2, and DL-*epi*-inosose-2, and its formation was accounted for by the reaction sequence shown (Scheme 46).

The n.m.r. spectra of various inositol osotriazoles have been examined and the results indicate that the majority of such compounds adopt a half-chair conformation (*e.g.* 164).[369]

The optical properties and structural changes of sugar osazones during mutarotation have been studied by n.m.r. and c.d.[370] An empirical rule has been developed to assist configurational assignments. The behaviour on protonation of diphenylformazans [*e.g.* D-mannose diphenylformazan (165)] and related compounds has been examined spectrophotometrically.[371] These compounds formed purple, blue, or green protonated cations, and the nature of the spectral changes suggested that the highly coloured cations have resonance-stabilised structures.

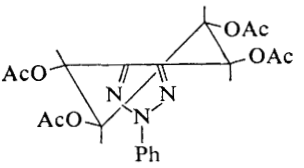
[365] H. S. Blair and G. A. F. Roberts, *J. Chem. Soc.* (*C*), 1969, 2357.
[366] W. S. Chilton, *J. Org. Chem.*, 1968, **33**, 4459.
[367] T. Ito, *Agric. and Biol. Chem.* (*Japan*), 1969, **33**, 1217.
[368] A. J. Fatiadi, *Carbohydrate Res.*, 1969, **9**, 177.
[369] A. J. Fatiadi, *Chem. and Ind.*, 1969, 617.
[370] L. Mester, *Chimia* (*Switz.*), 1969, **23**, 133.
[371] H. S. Isbell and A. J. Fatiadi, *Carbohydrate Res.*, 1969, **11**, 303.



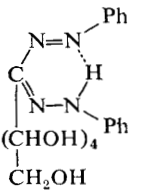
(163)

Reagents: i, ^{-}OAc; ii, $PhNHNH_2$, AcOH, O_2; iii, $PhNHNH_2$

Scheme 46



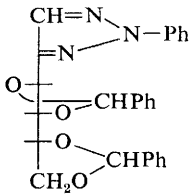
(164)



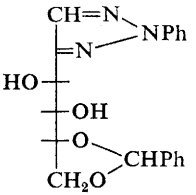
(165)

The zinc chloride-catalysed condensation of D-glucose phenylosotriazole and benzaldehyde has been studied in detail.[372] The 3,5:4,6-diacetal was not a product, since this compound would require a *trans*-decalin type structure with the bulky phenylosotriazole group axially substituted. The main product was the 3,4:5,6-diacetal (166) in which both acetal carbon atoms have the *R* configuration. Partial acidic hydrolysis of the 3,4:5,6-diacetal afforded the 3,4-acetal. The course of the condensation reaction was monitored by product analysis at intervals. It was found that the initially formed 5,6-acetal (167) isomerised to the 4,6-acetal (168) which then gave the 3,4:5,6 fully substituted product.

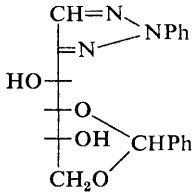
[372] D. J. Brecknell and R. M. Carman, *Austral. J. Chem.*, 1969, **22**, 1669.



(166)

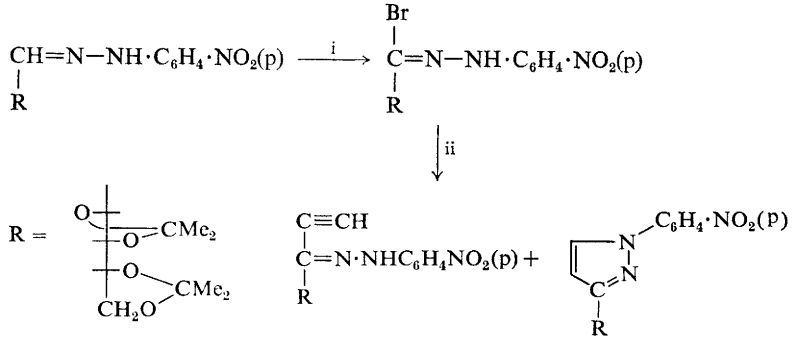


(167)



(168)

New compounds related to *C*-glycosides have been prepared from hydrazones of *aldehydo* sugar derivatives as illustrated [373] (Scheme 47).



Reagents: i, Br_2; ii, HC≡C·MgBr

Scheme 47

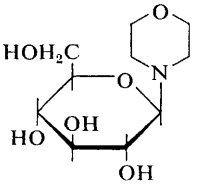
[373] J. M. J. Tronchet, A. Jotterand, and N. Le-Hong, *Helv. Chim. Acta*, 1969, **52**, 2569.

10

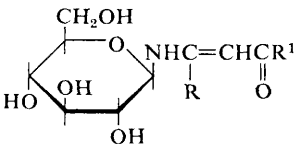
Miscellaneous Nitrogen-containing Compounds

Glycosylamines and Related Compounds

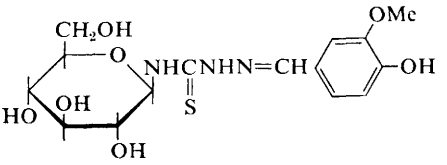
The synthesis of β-D-glucopyranosyl morpholine (169) has been described, and also its Amadori rearrangement to 1-deoxy-1-morpholino-D-fructose.[374] The condensation of β-diketones and glycosylamines gave the expected *N*-glycosyl derivatives such as (170).[375] The structures of some ethyl 3-(glycosylamino)crotonates have been studied by n.m.r. methods.[376]



(169)



(170)



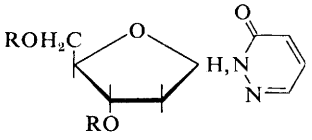
(171)

The mechanism of the acid-catalysed formation of *N*-arylglycosylamines has been investigated; a bimolecular mechanism was considered the most likely.[377,377a] The mutarotation of *N*-*p*-chlorophenylD-glucosylamine has been studied in methanol in the presence of carbobenzoxypeptides and the reaction used to find the acid strengths of the latter.[378] Studies on the mutarotation of *N*-*p*-nitrophenyl D-glucopyranosylamine in pyridine containing

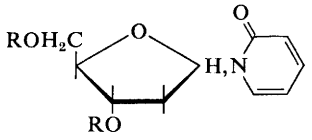
[374] R. Bognár, H. Frenzel, and I. Farkas, *Acta. Chim. Acad. Sci. Hung.*, 1969, **60**, 163.
[375] A. Gómez Sánchez, M. Tena Aldave, and J. Velasco Del Pino, *Carbohydrate Res.*, 1969, **10**, 19.
[376] A. Gómez Sánchez, M. Tena Aldave, and U. Scheidegger, *Carbohydrate Res.*, 1969, **9**, 335.
[377] V. A. Afanas'ev and V. A. Kharmats, *Zhur. fiz. Khim.*, 1969, **43**, 500.
[377a] V. A. Kharmats and V. A. Afanas'ev, *Zhur. fiz. Khim.*, 1968, **42**, 2078.
[378] T. Jasinski, K. Smiataczowa, T. Sokolowska, and J. Sokolowski, *Zeszyty Nauk., Mat., Fiz., Chem.*, 1967, **7**, 137 (*Chem. Abs.*, 1969, **70**, 4463*w*).

water, acetic acid, and labelled *p*-nitroaniline showed that base exchange occurred.[379] Periodate oxidation of *N*-*p*-methoxyphenyl β-D-glucopyranosylamine and related compounds showed that it was fully oxidised and that the liberated base also underwent reaction. The maximum uptake was 7 moles of oxidant.[380]

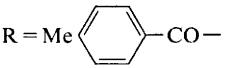
Syntheses of *N*-*p*-nitrophenyl glycosylamines of cellobiose, lactose, and maltose have been described.[381] The conformations of the aglycones in acetylated *N*-(β-D-glucopyranosyl)-pyridones, and in aza analogues, have been studied by n.m.r. methods.[382] Condensation of acetohalogeno-sugars with imidazole gave the corresponding glycosyl imidazoles, which were assumed to be β-derivatives from their o.r.d. spectra.[383] Glycopyranosyl thiosemicarbazones of aromatic aldehydes such as (171) have been described.[384] The ammonolysis of sugar esters has been shown to yield amongst other products *N*-acylaldosylamines.[232]



(172)



(173)



It has been shown that the pyridazones (172) obey Hudson's rules, but for the pyridones (173) they are reversed.[385]

Azides

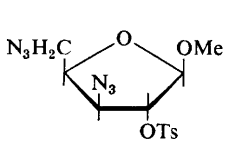
Many papers reported in Chapter 8 used azides as intermediates in the synthesis of amino-sugars; they will not be referred to again here.

ω-Azido-ω-deoxy-derivatives of D-glucose, -mannose, -galactose, and L-arabinose dithioacetals have been prepared *via* the ω-toluene-*p*-sulphonates.[386] Crystalline 3-azido-3-deoxy-D-altrose, the first free azido-sugar, has been prepared from methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altroside by removal of the blocking groups. The n.m.r. spectrum of the

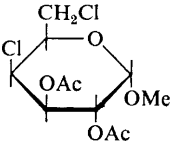
[379] Z. Pawlak and E. Górska, *Roczniki Chem.*, 1969, **43**, 1241.
[380] Z. Fialkiewiczowa and J. Sokolowski, *Zeszyty Nauk., Mat., Fiz., Chem.*, 1967, **7**, 131 (*Chem. Abs.*, 1968, **69**, 97,062*g*).
[381] S. Adachi, *Carbohydrate Res.*, 1969, **10**, 165.
[382] P. Nuhn, A. Zschunke, and G. Wagner, *Z. Chem.*, 1969, **9**, 335.
[383] J. Jasińska and J. Sokolowski, *Roczniki Chem.*, 1969, **43**, 867.
[384] W. Wieniawski and C. Gmernicka-Haftek, *Diss. Pharm. Pharmacol.*, 1968, **20**, 411 (*Chem. Abs.* 1969, **70**, 58,174).
[385] P. Nuhn, A. Zschunke, D. Heller, and G. Wagner, *Tetrahedron*, 1969, **25**, 2139.
[386] J. Fernández-Bolaños and R. Guzmán de Fernández-Bolaños, *Anales de Quim.*, 1969, **65**, 415.

product (in DMSO) suggested that it existed in the α-furanose form.[387] The same group have described the synthesis of 2- and 3-azido-deoxy-derivatives of 1,6-anhydro-β-D-altropyranose by treatment of the corresponding methyl α-glycosides with toluene-*p*-sulphonic acid in benzene.[388]

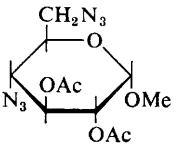
Methyl 3,5-diazido-3,5-dideoxy-2-*O*-toluene-*p*-sulphonyl-β-D-xylofuranoside (174) has been prepared by azidolysis of the corresponding 5-azido-5-deoxy-2,3-di-*O*-sulphonyl compound; as expected the C-2-OTs group could not be displaced.[389] Chloro-deoxy-derivatives (prepared *via* reactions with sulphuryl chloride) have been shown to be useful intermediates for the synthesis of azido-sugars. Thus, (175) gave (176) in 90% yield. Reaction with (177) gave the required azide (178) together with (179).



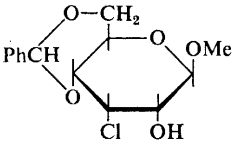
(174)



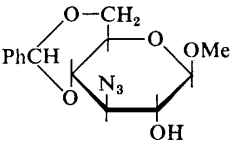
(175)



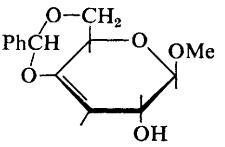
(176)



(177)



(178)

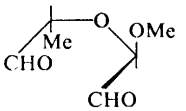


(179)

The o.r.d. spectra of a number of 4-azido-pentopyranosides have been investigated (see p. 183).[390]

Nitro-compounds

Baer has reviewed compounds of this type.[390a] The nitromethane cyclisation of the dialdehyde (180) (from periodate oxidation of methyl α-L-rhamnopyranoside) has been reinvestigated with the intention of providing more

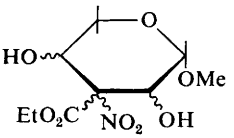


(180)

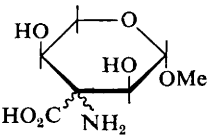
[387] H. Kuzuhara, K. Yakabi, H. Ohrui, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 285.
[388] H. Kuzuhara, H. Ohrui, and S. Emoto, *Carbohydrate Res.*, 1969, **11**, 9.
[389] J. Hildesheim, J. Cléophax, A. M. Sépulchre, and S. D. Géro, *Carbohydrate Res.*, 1969, **9**, 315.
[390] B. T. Lawton, W. A. Szarek, and J. K. N. Jones, *Chem. Comm.*, 1969, 787.
[390a] H. H. Baer, *Adv. Carbohydrate Chem.*, 1969, **24**, 67.

information on the course of such reactions.[391] Four crystalline, 3,6-dideoxy-3-nitro-α-L-hexopyranosides were obtained in a total yield of 75%. These had the *gluco*, *manno*, *talo*, and *galacto* configurations (18 : 8 : 3 : 1). Shortening the reaction time led to a ratio of 8 : 8 : 2·3 : 1·7.

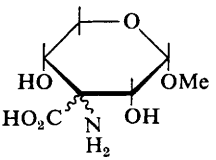
The cyclisation reaction has been extended to the use of ethyl nitroacetate which has been reacted with the dialdehydes from methyl β-L-arabinopyranoside and β-D-xylopyranoside. A mixture of four products resulted, all with the general methyl 3-deoxy-3-*C*-ethoxycarbonyl-3-nitro-pentopyranoside structure (181). The stereochemistry of the products was investigated by n.m.r. studies on the derived amino-acids. Further, chloramine-T oxidation of the amino-acids gave the corresponding 3-uloses. Of the four compounds, two gave the same 3-ulose and were therefore epimeric at C-3; from n.m.r. these had structure (182). The other two were (183) and (184), the major components of the mixture.[392]



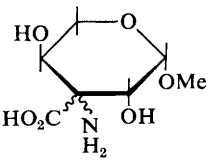
(181)



(182)



(183)

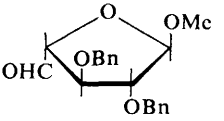


(184)

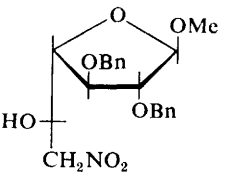
Addition of nitromethane, in the presence of sodium methoxide, to methyl 2,3-di-*O*-benzyl-α-L-*arabino*-pentodialdo-1,4-furanoside (185) gave a mixture of the L-*altro* (186) and D-*galacto* (187) products (C-5 epimers) in a ratio of 2·5 : 1, a result that was rationalised in stereochemical terms. In further work, addition was made to 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanoside (188); the main product was the 6-nitro-*gluco* compound.[393]

Addition of nitryl iodide (INO_2) to (189), followed by sodium borohydride reduction of the product, gave (190) which on acid hydrolysis gave 3-deoxy-3-*C*-nitromethyl-D-allose, that existed predominantly in the β-furanose form.[394] The reaction between 3-uloses and nitromethane has

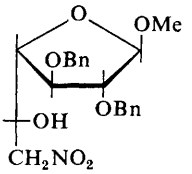
[391] H. H. Baer and K. Čapek, *Canad. J. Chem.*, 1969, **47**, 99.
[392] H. Yanagisawa, M. Kinoshita, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1969, **42**, 1719.
[393] H. Saeki, T. Iwashige, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 2410.
[394] W. A. Szarek, J. S. Jewell, I. Szczerek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 4473.



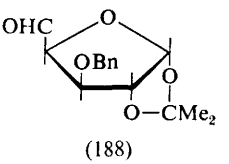
(185)



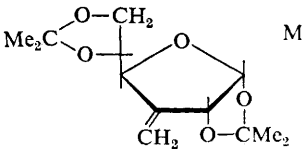
(186)



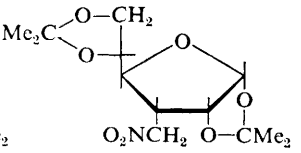
(187)



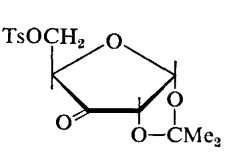
(188)



(189)



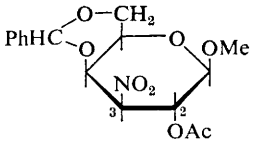
(190)



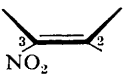
(191)

been shown to give novel branched-chain nitro-sugars,[277] *e.g.* (191) gave (67).

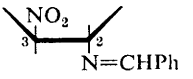
Many reactions of nitro-sugars have been studied including their use in the synthesis of lincosamine (see p. 152). Treatment of (192) with aqueous ammonia did not lead to a 2-amino-2-deoxy-product (expected on the basis of an elimination–addition), but similar treatment of (193) gave (194), in which the benzylidene group at C-2-N arose from decomposition of some of the starting compound (193). A more efficient procedure for the conversion of (193) into (194) was by the use of ammonium acetate and acetamide. Treatment of (194) with acetic anhydride gave (195), from which a series of derivatives of 2,3-diamino-2,3-dideoxy-galactose were prepared.[395]



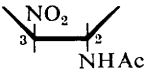
(192)



(193)



(194)

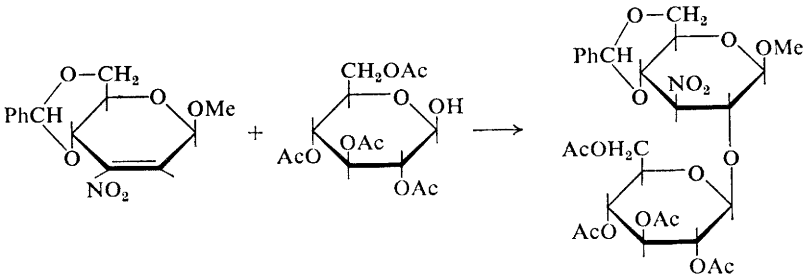


(195)

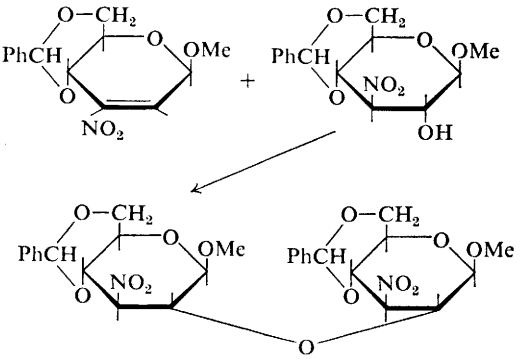
Nitro-compounds have been converted into unsaturated lactones (see p. 115) and into biglycosylamines.[358] The addition reactions of unsaturated nitro-compounds have been extended to the synthesis of novel di-

[395] H. H. Baer and K. S. Ong, *J. Org. Chem.*, 1969, **34**, 560.

saccharide derivatives as in Scheme 48. Use of the α-anomer of the nitro-compound gave an analogous product but in lower yield.[396] Bis-glycosidyl ethers were also prepared (Scheme 49) where the glycosidic



Scheme 48



Scheme 49

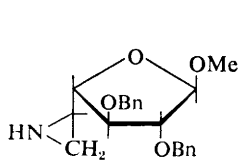
bonds were αα, αβ, βα, and ββ. In all cases the products had the *gluco* configuration.

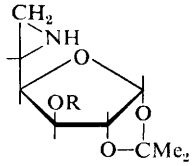
Epimino-sugars

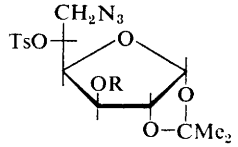
Several papers have appeared by Saeki and Ohki on 5,6-epimino-compounds. Full details (*cf.* vol. 2, p. 104) of the synthesis of methyl 2,3-di-*O*-benzyl-5,6-dideoxy-5,6-epimino-α-L-altrofuranoside (196) and of 3-*O*-benzyl-5,6-dideoxy-5,6-epimino-1,2-*O*-isopropylidene-β-L-idofuranose (197) (R = Bn) have been reported.[397] Ring-opening of either epimine with azide ion or with acetate ion occurred exclusively at C-6.

Formation of compounds (197) from system (198) has been further studied, and the yields (*via* LAH reduction) were much lower for R = H

[396] H. H. Baer and F. Kienzle, *Canad. J. Chem.*, 1969, **47**, 2819.
[397] H. Saeki and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 2471.


(196)

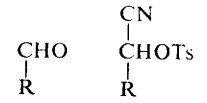

(197)


(198)

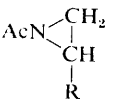
than for R = $SiMe_3$, Ac, or Bn, suggesting that in the hydroxy-compound that group competed with the 6-amino-group in the displacement of the 5-sulphonyloxy-group.[398] In contrast to results reported in the steroid series, reduction of (198) (R = Ac) with sodium borohydride-tris(α,α′-dipyridyl)cobalt(II) bromide gave the 6-amino-5-*O*-sulphonyl derivative, but with some acetate hydrolysis.

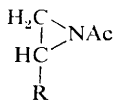
The full details of the synthesis of nojirimycin *via* 3-*O*-benzyl-5,6-dideoxy-5,6-epimino-1,2-*O*-isopropylidene-α-D-glucofuranose have been reported [399] (see vol. 2, p. 119).

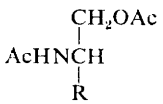
Treatment of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose (199) with sodium cyanide in aqueous methanol gave a mixture of cyanohydrins that were separated as their 6-*O*-toluene-*p*-sulphonyl esters (200). Reduction of the cyano-sulphonates gave the 6,7-dideoxy-6,7-epimino-heptoses, isolated as their *N*-acetyl derivatives (201) and (202), both of which underwent ring-opening at C-7 with acetic acid to give the 6-acetamido-heptose derivatives (203) and (204).[400]

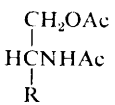

(199)

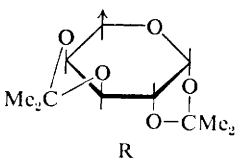
(200)


(201)


(202)


(203)


(204)



A 5,6-epimino-compound was believed to occur as an intermediate in the synthesis of (124).[316] Full details have appeared of the reaction of 5,6-di-*O*-methanesulphonyl compounds with hydrazine which yielded 5,6-(*N*-amino)epimines [401] (*cf.* vol. 2, p. 105).

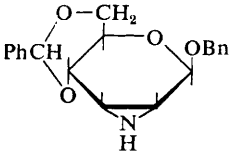
Reaction of compounds (205), (206), or (207) with sodium isopropoxide gave only the 2,3-epimine (208), but when a weak base was used, such as

[398] H. Saeki and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1664.
[399] H. Saeki and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 2477.
[400] H. Saeki and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1974.
[401] H. Paulsen and D. Stoye, *Chem. Ber.*, 1969, **102**, 820.

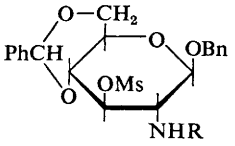
potassium acetate in aqueous 2-ethoxyethanol, oxazolines and oxazolidones were formed.[402]

A preliminary report has appeared on the synthesis of a series of methyl 2,3-deoxy-2,3-epimino-β-D-lyxofuranosides. Azidolysis of (209) gave two azidobenzamido-derivatives, of which the major one was believed to have the *arabino* configuration.[403]

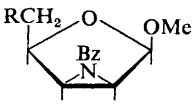
The synthesis of the related 2,3-epimine (210) has been described and also its similar azidolysis.[389] Synthesis of epimino-derivatives of the general formula (211) have been described [404] some of which have been reported previously in a preliminary communication (see vol. 1, p. 71). All were made from azido-sulphonate precursors.



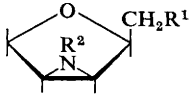
(208)



(205) R = Bz
(206) R = Ac
(207) R = Cbz



(209) R = OH
(210) R = NHBz



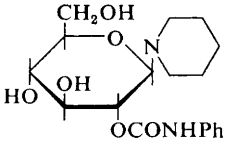
(211)

Heterocyclic Derivatives

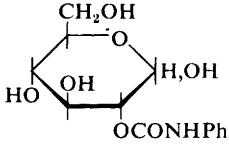
Treatment of a variety of glycosyl azides in refluxing toluene with phenylacetylene gave mixed *N*-triazole derivatives by 1,3-dipolar additions. N.m.r. spectroscopy was used to determine the structures and the anomeric configurations of the products.[405]

Acid hydrolysis of (212) gave a product previously reported to have structure (213), but now shown to be structure (214). Compound (213) was the first hydrolysis product which was then slowly converted into (214). Increase in the reaction temperature, or in the acid concentration, caused an increase in the proportion of a third product (215), which could also be formed directly from (214) by acid treatment. Alkaline conditions favoured the conversion of (213) into (214).[406]

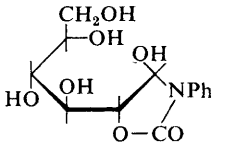
[402] W. D. Rhoads and P. H. Gross, *Carbohydrate Res.*, 1969, **11**, 561.
[403] J. Cléophax, S. D. Géro, J. Hildesheim, R. D. Guthrie, and C. W. Smith, *Chem. and Ind.*, 1969, 784.
[404] J. Cléophax, J. Hildesheim, A.-M. Sépulchre, and S. D. Géro, *Bull. Soc. chim. France*, 1969, 153.
[405] M. T. García-López, G. Garcia-Muñoz, J. Iglesias, and R. Madroñero, *J. Heterocyclic Chem.*, 1969, **6**, 639.
[406] W. E. Dick, D. Weisleder, and J. E. Hodge, *J. Org. Chem.*, 1969, **34**, 2654.



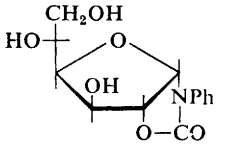
(212)



(213)

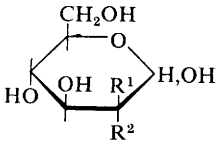


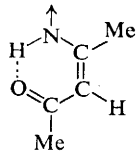
(214)



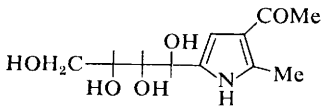
(215)

Condensation of 2-amino-2-deoxy-D-glucose or -D-mannose with 2,4-pentanedione in alkaline solution gave the derivatives (216) and (217), which underwent cyclisation to the substituted pyrrole derivatives (218).[407] Detailed 220 MHz n.m.r. spectra of (216) and (217) were reported.



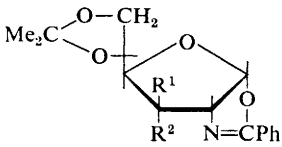
(216) $R^1 = H$, $R^2 =$ 

(217) $R^2 = H$, $R^1 =$

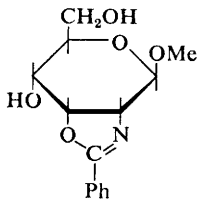


(218)

Oxazolines have been used in the synthesis of aminodisaccharides;[95] the mass spectra of 2-methyl- and 2-phenyl-glyco[1^1,2^1:4,5]-2-oxazolines have been studied (see p. 179). The methanolysis of oxazolines (218A) and (218B) has been reinvestigated and found to give mixtures of 2-benzamido-2-deoxy-hexosides in the pyranoid and furanoid form—(218B) also gave



(218A) $R^1 = OH$, $R^2 = H$
(218B) $R^1 = H$, $R^2 = OH$



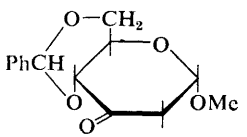
(218C)

[407] N. S. Bhacca and J. J. Ludowieg, *Carbohydrate Res.*, 1969, **11**, 432.

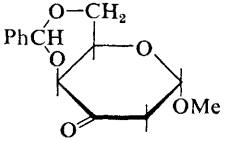
(218C) in which the oxazoline ring had migrated.[407a] A comment on this paper has been made by Micheel.[407b]

Other Nitrogen-containing Compounds

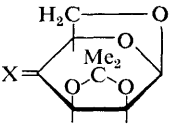
Oximes of the two 3-uloses (219) and (220) have been prepared; the former gave only one isomer (*syn*), the latter both *syn* and *anti* forms, the structures of which were assigned by n.m.r. spectroscopy.[313] Treatment of the keto-sugar (58) with hydroxylamine hydrochloride in aqueous pyridine



(219)

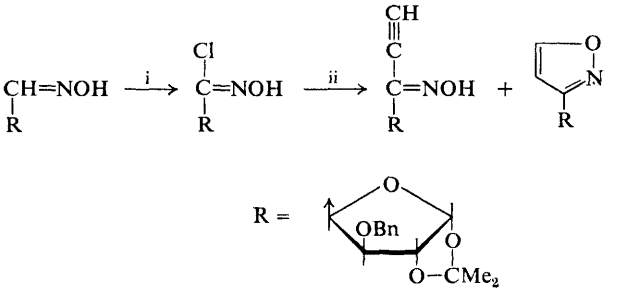


(220)



(221) X = O
(222) X = N_2

gave a 91% yield of the pyridinium sulphonate (59), which underwent facile displacement reactions at C-2.[271] The reactions between sugar oximes and acetylenic Grignard reagents have been examined;[373] for example, see Scheme 50.



Reagents: i, Cl_2; ii, CH≡CMgBr

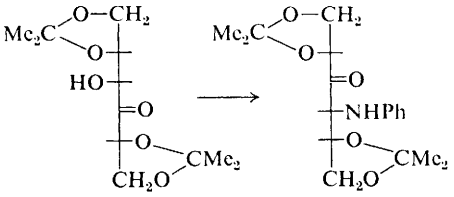
Scheme 50

The diazo-sugar, 1,6-anhydro-4-deoxy-4-diazo-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranose (221), has been synthesised from the corresponding keto-sugar (222) *via* its 2,4,5-trichlorobenzenesulphonylhydrazone. Compound (221) was a stable yellow crystalline solid and should be a useful synthetic intermediate.[408]

Branched-chain sugar derivatives bearing a cyano-group have been prepared, (see p. 116) and a glycosyl cyanide has been used in the synthesis of 2,5-anhydro-allonic acid (see p. 135).

[407a] W. Meyer zu Reckendorf, N. Wassiliadou-Micheli, and D. Delevalle, *Chem. Ber.*, 1969, **102**, 1076.
[407b] F. Micheel, *Chem. Ber.*, 1969, **102**, 2880.
[408] D. Horton and E. K. Just, *Chem. Comm.*, 1969, 1116.

The Amadori rearrangement has been shown to be catalysed by Lewis acid complexes; see, for example, the rearrangement shown in Scheme 51.[409]



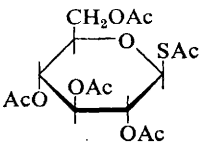
Scheme 51

[409] J. Yoshimura, M. Funabashi, and H. Simon, *Carbohydrate Res.*, 1969, **11**, 276.

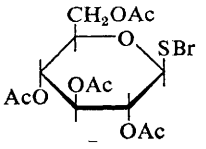
11
Thio-sugars*

Chemical and biochemical aspects of thio-sugars have been reviewed (in Japanese).[409a]

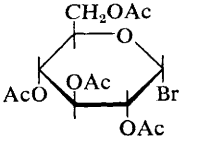
The reaction of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (222) with bromine has been studied. Under controlled conditions in carbon tetrachloride, the sulphenyl bromide (223) was formed, but prolonged reaction times gave the glucosyl bromide (224). With



(222)



(223)

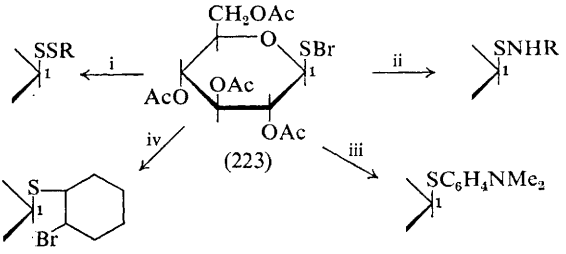


(224)

R—S—S—R R = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl

(225)

bromine in chloroform (222) gave (225), whilst (223) was converted into (225) on heating in ethanol.[410] In a further paper, the reactions of (223) with a variety of reagents were explored (Scheme 52).[411]



Reagents: i, RSH; ii, RNH_2; iii, $PhNMe_2$; iv, cyclohexene

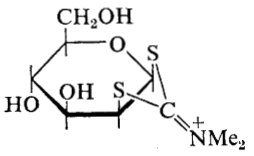
Scheme 52

[409a] S. Tejima, *Seikagaku*, 1967, **39**, 897 (*Chem. Abs.*, 1969, **70**, 38,008*s*).
[410] R. H. Bell and D. Horton, *Carbohydrate Res.*, 1969, **9**, 187.
[411] R. H. Bell, D. Horton, and M. J. Miller, *Carbohydrate Res.*, 1969, **9**, 201.

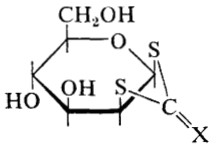
* See also Section 12.

Reaction between methyl 2-bromo-2-deoxy-β-D-glucopyranoside and potassium thiomethoxide has been shown to give methyl 3-*S*-methyl-3-thio-β-D-altropyranoside, presumably *via* a 2,3-epoxide intermediate.[412]

The heterocyclic compound (227) has been prepared by the action of methanolic ammonia on the salt (226). Its structure was established by conversion into *N*-substituted derivatives and into the trithiocarbonate (228) by hydrogen sulphide. Treatment of (228) with triphenylphosphite and acetylation gave tri-*O*-acetyl-D-glucal.[413]

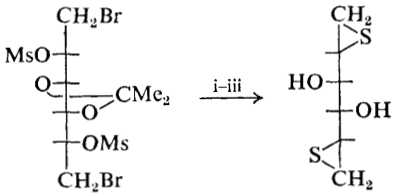


(226)



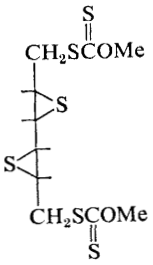
(227) X = NH
(228) X = S

1,2:5,6-Diepithio-L-iditol has been synthesised as shown in Scheme 53. The di-*O*-methanesulphonate of (229) gave (230) when reacted with carbon



(229)

Reagents: i, NaSBz; ii, NaOMe; iii, H^+



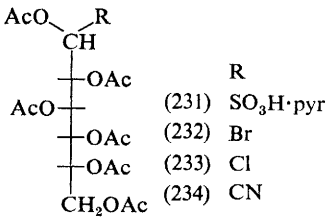
(230)

Scheme 53

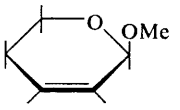
[412] T. van Es, *Carbohydrate Res.*, 1969, **11**, 282.
[413] S. Ishiguro and S. Tejima, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 2040.

disulphide in the presence of sodium methoxide.[414] Hydrogen peroxide oxidation of 1-*S*-ethyl-1-thio-L-galactitol gave two isomeric sulphoxides; attempts to carry out intramolecular DMSO-type oxidations were unsuccessful [415] (*cf.* vol. 2, p. 144). 1-Thio-D-glucitol and its disulphide have been prepared by the reaction of D-glucose and hydrogen sulphide in aqueous solution at elevated temperatures and pressures.[416]

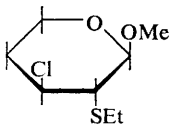
A series of aldose derivatives, *e.g.* (231), have been prepared and provide a new and potentially valuable means of obtaining acyclic sugar derivatives. Thus, D-glucose on treatment with cyclohexylamine bisulphite gave a crystalline adduct that on acetylation with acetic anhydride in pyridine gave (231), also prepared by an alternative synthesis *via* penta-*O*-acetyl-*aldehydo*-D-glucose. On treatment with phosphorus pentabromide and acetyl bromide, with phosphorus pentachloride, or with sodium cyanide (231) gave (232), (233), or (234) respectively, each of which could be converted into the *aldehydo*-sugar.[417]



	R
(231)	SO_3H·pyr
(232)	Br
(233)	Cl
(234)	CN



(235)



(236)

Formation of isopropylidene derivatives of D-ribose diethyl dithioacetal [197] and of bisulphite derivatives of D-glucose [199] has been described.

Treatment of the model unsaturated carbohydrate (235) with ethanesulphenyl chloride gave (236).[418] The synthesis of 5′-thio-adenosine and -uridine has been described.[419]

A further report (*cf.* vol. 1, p. 115) on the anti-tumour activity of sulphur-containing carbohydrate derivatives has appeared. Forty compounds of various classes were screened.[420]

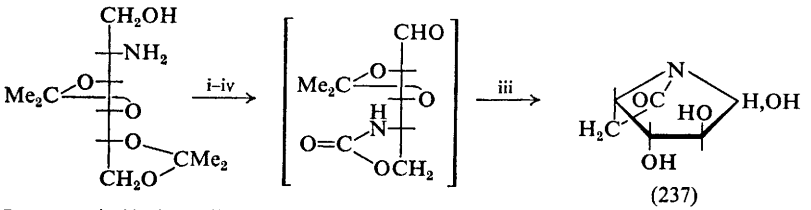
[414] J. Kuszmann and L. Vargha, *Carbohydrate Res.*, 1969, **11**, 165.
[415] R. J. Ferrier and D. T. Williams, *Carbohydrate Res.*, 1969, **10**, 157.
[416] A. R. Procter and R. H. Wiekenkamp, *Carbohydrate Res.*, 1969, **10**, 459.
[417] D. L. Ingles, *Chem. and Ind.*, 1969, 50.
[418] M. J. Baldwin and R. K. Brown, *Canad. J. Chem.*, 1969, **47**, 3553.
[419] A. M. Yurkevich, A. A. Amagaeva, I. P. Rudakova, and N. A. Preobrazhenskii, *Zhur. obshchei. Khim.*, 1969, **39**, 434.
[420] Y. Hasegawa, H. Kawasaki, S. Ishiguro, T. Maki, and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1739.

12

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

Nitrogen Derivatives

Paulsen and his co-workers have continued their activity in this field during 1969. Bicyclic derivatives of 4-amino-4-deoxy-L-xylose have been prepared as illustrated in Scheme 54.[421] 4-Amino-4-deoxy-L-xylose was prepared [422]

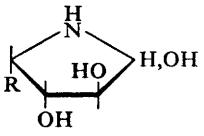


(237)

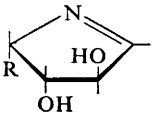
Reagents: i, $ClCO_2Et$; ii, NaOMe; iii, H^+; iv, IO_4^-

Scheme 54

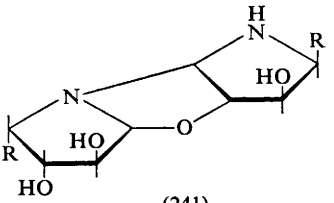
from the 1,2-isopropylidene acetal of (237), and 4-amino-4,5-dideoxy-L-xylose by a standard route from 5-deoxy-2,3-*O*-isopropylidene-D-arabinose diethyl acetal. The two amino-sugars (238) and (239) each existed in solution as an equilibrium mixture of the two forms (240) and (241) in



(238) R = Me
(239) R = CH_2OH



(240)



(241)

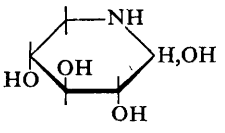
which the dimeric species predominated. These were characterised by cleavage to monomeric species on reaction with hydrogen, sulphur dioxide, or hydrogen cyanide, but were remarkably stable to acid (*cf.* vol. 2, p. 118).

[421] H. Paulsen, J. Brüning, and K. Heyns, *Chem. Ber.*, 1969, **102**, 459.
[422] H. Paulsen, K. Propp, and J. Brüning, *Chem. Ber.*, 1969, **102**, 469.

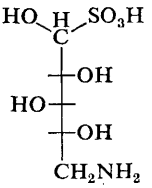
Similar equilibrium mixtures have been reported for 4-amino-4-deoxy-D-glucose and -D-galactose.[423]

All four 5-benzyloxycarbonylamino-5-deoxy-D-pentoses have been prepared and found to exist preponderantly in the six-membered ring form. Thus, an *N*-heterocyclic ring, which was shown by n.m.r. methods to adopt the chair conformation, was preferred to a furanoid ring. The α-D-*xylo*-, α-D-*lyxo*-, β-D-*arabino*-, and α- and β-D-*ribo*-isomers were isolated. In each case the molecules adopted chair conformations in which the C-1 hydroxy-group was situated axially, and the anomeric effect in such compounds was discussed. The *N*-acetylated monosaccharides with nitrogen in the ring had optical rotations which did not conform with Hudson's rules.[424]

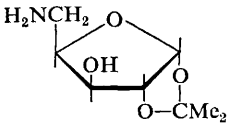
All four possible pentose analogues (*e.g.* 242) have been prepared from the corresponding bisulphite adducts (*e.g.* 243) which in turn were synthesised from the 5-amino-5-deoxy-1,2-*O*-isopropylidene pentofuranose derivatives (*e.g.* 244). The products existed in equilibrium with the tetrahydropyridine derivatives, *i.e.* (242) with (245). In acid solution, they rearranged to the Amadori products (*e.g.* 246) and also gave 3-hydroxypyridine (247). The magnitudes of the Cotton effects of compounds of



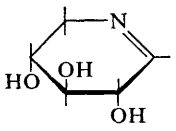
(242)



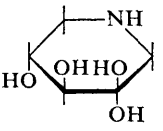
(243)



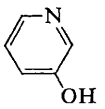
(244)



(245)



(246)



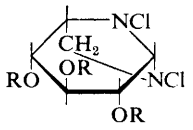
(247)

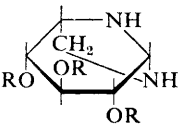
type (245) were discussed, and the o.r.d. curves of the saturated compounds [(242) and its three isomers] were found to be similar to those of the pentoses.[425] Hudson's isorotation rules were shown to apply for the saturated compounds without *N*-substituents.

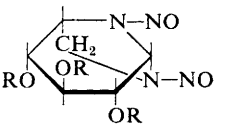
The product, (248), of the reaction between (249) and sodium hypochlorite, together with intermediate monochlorinated products, has been

[423] H. Paulsen, K. Propp, and K. Heyns, *Tetrahedron Letters*, 1969, 683.
[424] H. Paulsen and F. Leupold, *Chem. Ber.*, 1969, **102**, 2804.
[425] H. Paulsen and F. Leupold, *Chem. Ber.*, 1969, **102**, 2822.

examined by n.m.r. spectroscopy. Nitrosation of (248) afforded (250), the stereochemistry of which was considered in detail.[426]

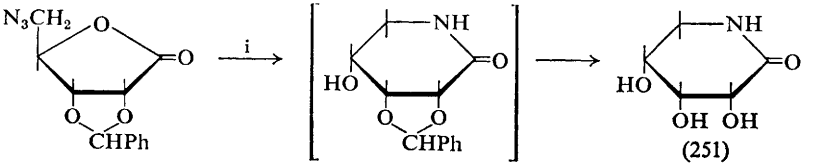

(248)


(249)


(250)

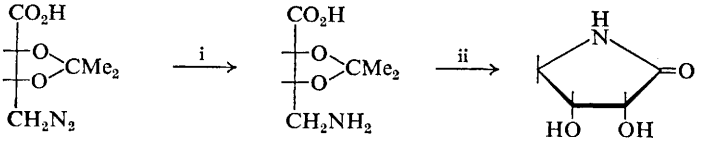
(R = TMS)

A series of sugar lactams having 6- and 7-membered rings was prepared [427] by reductive cyclisation of azido lactones (for example, Scheme 55); 5-membered ring compounds have also been prepared from acids as shown in Scheme 56. The reaction of the tri-*O*-acetyl derivative of (251)


(251)

Reagents: i, H_2–Pd

Scheme 55

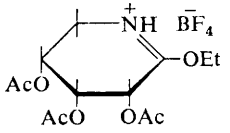

(252)

Reagents: i, H_2–Pd; ii, H^+

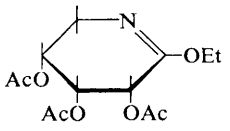
Scheme 56

with triethyloxonium fluoroborate gave (253) which, with weak base, produced (254), a lactam ether which was a new type of carbohydrate derivative. Compound (252) was prepared from 2,3-*O*-isopropylidene-D-erythronolactone by reaction with sodium azide in DMF, a new reaction for the introduction of functional groups into carbohydrates. However, efforts to apply this reaction to the formation of lactams from secondary alcoholic groups were unsuccessful.[427] Compound (255), formed from D-mannitol by successive periodate oxidation and chromium trioxide oxidation of its 1-azido-3,4-dideoxy-derivative, was converted stereospecifically into *S*(−)-piperidin-3-ol by reductive cyclisation and LAH reduction.[428]

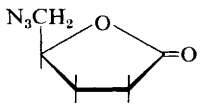
[426] H. Paulsen and U. Grage, *Chem. Ber.*, 1969, **102**, 3854.
[427] S. Hanessian, *J. Org. Chem.*, 1969, **34**, 675.
[428] C. C. Deane and T. D. Inch, *Chem. Comm.*, 1969, 813.



(253)

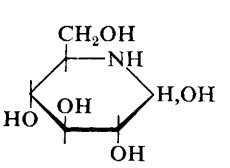


(254)

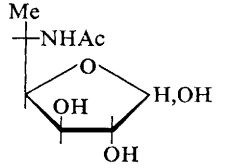


(255)

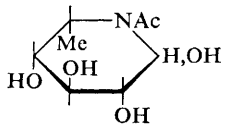
Full details of the synthesis of 3-*O*-benzyl-5,6-dideoxy-5,6-epimino-1,2-*O*-isopropylidene-α-D-glucofuranose [399] and its conversion into 5-amino-5-deoxy-D-glucose (nojirimycin) (256) have been published (*cf.* vol. 2, p. 119).



(256)



(257)

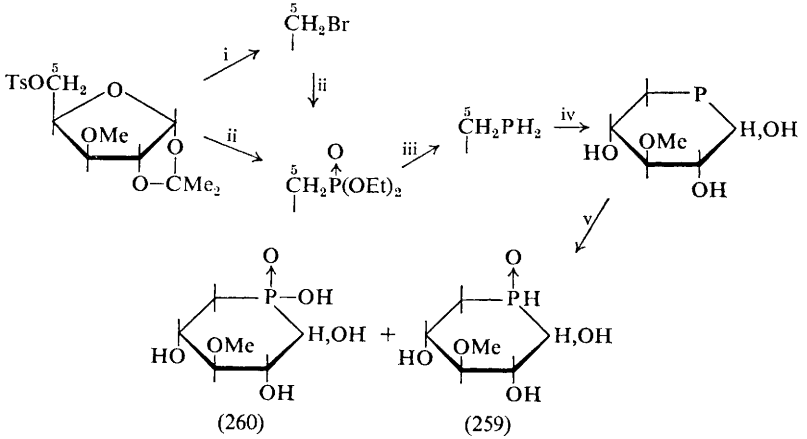


(258)

5-Acetamido-5,6-dideoxy-L-idose has been synthesised and shown to exist in solution both in the furanose form (257) and in the piperidinose form (258).[316] A branched-chain compound, an analogue of apiose, has been synthesised with nitrogen as the ring hetero-atom.[428a]

Phosphorus Derivatives

Xylose derivatives with phosphorus in the ring have been prepared [429] according to Scheme 57.



(260) (259)

Reagents: i, Et_4NBr; ii, $P(OEt)_3$; iii, LAH; iv, HCl; v, O_2

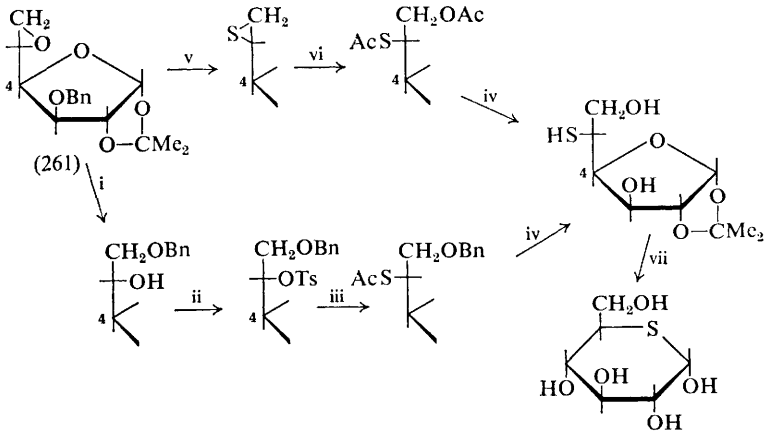
Scheme 57

[428a] M. H. Halford, D. H. Ball, and L. Long jun., *Chem. Comm.*, 1969, 255.
[429] R. L. Whistler and C.-C. Wang, *J. Org. Chem.*, 1968, **33**, 4455.

The products were characterised by n.m.r. spectroscopy. Products (259) and (260) consumed 3 and 2 moles of periodate respectively, 1 mole of oxidant with (259) being used in oxidation of the phosphine. Obviously, α-hydroxyphosphinic acids are cleaved by periodate.

Sulphur Derivatives

Two methods (Scheme 58) have been developed[430] for the synthesis of 5-thio-D-glucose in greatly improved yield from (261), which itself has also been prepared more conveniently than by previously described routes.



Reagents: i, NaOBn; ii, TsCl, pyr; iii, KSAc; iv, Na–NH_3(liq); v, $CS(NH_2)_2$; vi, KOAc, HOAc, Ac_2O; vii, H^+

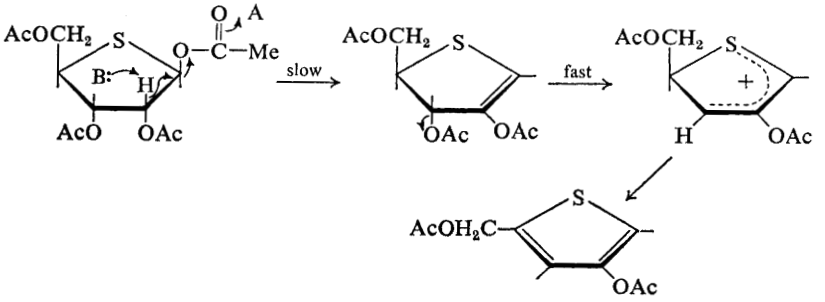
Scheme 58

A thiophen derivative has been reported[431] as the major product formed when 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose was heated in DMF in the presence of acid and base; the sequence outlined in Scheme 59 was suggested.

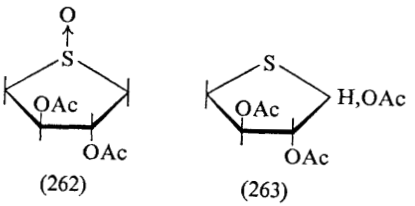
A Pummerer-type rearrangement of the sulphoxide (262) to give 1,2,3-tri-*O*-acetyl-4-thio-DL-threofuranose (263) has been reported.[432] The *erythro*-analogue of (263) was similarly prepared.

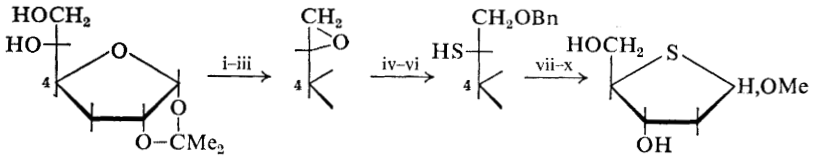
Both anomers of methyl 2-deoxy-4-thio-D-*erythro*-pentofuranosides have been synthesised as illustrated in Scheme 60.[269] The starting material was prepared from 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose by reaction with potassium thiolacetate in DMF, selective hydrolysis, and desulphurisation.

[430] U. G. Nayak and R. L. Whistler, *J. Org. Chem.*, 1969, **34**, 97.
[431] R. L. Whistler and D. J. Hoffman, *Carbohydrate Res.*, 1969, **11**, 137.
[432] J. E. McCormick and R. S. McElhinney, *Chem. Comm.*, 1969, 171.



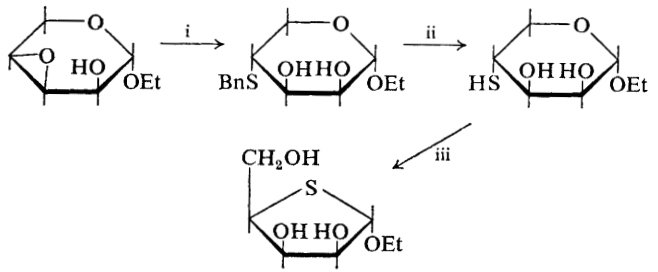
Scheme 59





Reagents: i, BzCl, $CHCl_3$, pyr; ii, TsCl, $CHCl_3$, pyr; iii, MeOH, MeONa; iv, NaOBn, BnOH; v, TsCl, $CHCl_3$, pyr; vi, KSAc, DMF; vii, aq AcOH; viii, IO_4^-; ix, MeOH, HCl*; x, Na, NH_3(liq)

Scheme 60



Reagents: i, NaSBn; ii, Na–NH_3(liq); iii, EtOH, H^+

Scheme 61

* Anomers separated before debenzylation.

The synthesis of ethyl 4-thio-α-D-lyxofuranoside has been achieved [433] by the sequence shown in Scheme 61; the methyl glycoside was also prepared as was the β-D-*ribo*-analogue from the known 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose.

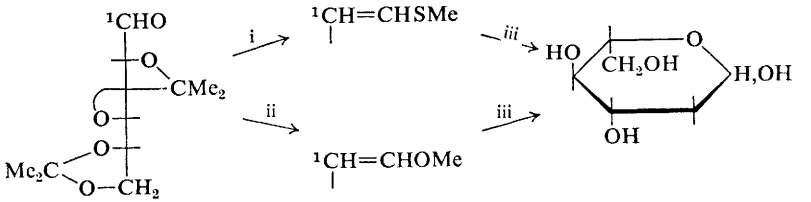
[433] J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, 1969, **34**, 2643.

13
Deoxy-sugars

A wide variety of pyranoid deoxy-sugar derivatives have been synthesised in Lemieux's laboratory for the purposes of investigating their solvation and ring conformations in solution (Chapter 23). Others have been prepared from the halogenated compounds mentioned in Chapter 7.

The reaction of benzylidene acetals with *N*-bromosuccinimide, which offers a means of preparing bromodeoxy compounds and thence deoxy derivatives, has been extensively examined,[194–196] and has been applied to methyl 4,6-*O*-benzylidene-2-*O*-benzoyl-3-deoxy-α-D-*ribo*-hexopyranoside in a synthesis of 3,6-dideoxy-D-*ribo*-hexose [300] (see also p. 35).

A booklet has appeared on the biosynthesis of 2-deoxy-D-*erythro*-pentose,[434] and new chemical syntheses have been described for 2-deoxy-aldoses, based on Wittig reaction products, as is illustrated in Scheme 62



Reagents: i, $Ph_3P{=}CHSMe$;[435] ii, $Ph_3P{=}CHOMe$;[436] iii, H^+

Scheme 62

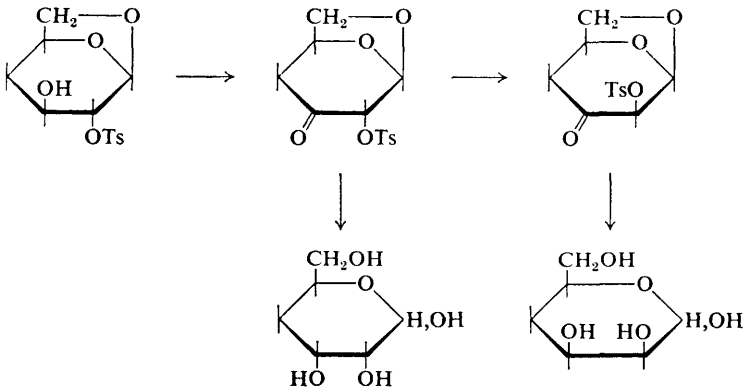
for 2-deoxy-L-*arabino*-hexose.[435, 436] Russian workers have reported on the same method, but using alkoxymethylenephosphoranes.[437]

Acid hydrolysis of 3,4,6-tri-*O*-acetyl-2-chloro-2-deoxy-β-D-mannopyranosyl chloride caused elimination of chlorine, and addition of water to the glycal intermediate to give 2-deoxy-D-*arabino*-hexose.[286] 2-Deoxy-D-*gluco*-heptose has been prepared by standard procedure from D-glucose,[14] and an improved method for synthesising methyl 2-deoxy-α-D-*arabino*-hexofuranoside has already been referred to.[69] The e.s.r. spectrum of

[434] P. Reichard, 'The Biosynthesis of Deoxyribose,' John Wiley, New York, 1968.
[435] J. M. J. Tronchet, S. Jaccard-Thorndahl, and Br. Baehler, *Helv. Chim. Acta*, 1969, **52**, 817.
[436] J. M. J. Tronchet, E. Doelker, and Br. Baehler, *Helv. Chim. Acta*, 1969, **52**, 308.
[437] Yu. A. Zhdanov and V. G. Alexeeva, *Carbohydrate Res.*, 1969, **10**, 184.

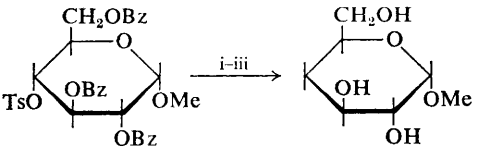
γ-irradiated single crystals of 2-deoxy-D-*erythro*-pentose has been examined.[438] The synthesis of both anomers of methyl 2-deoxy-4-thio-D-*erythro*-pentofuranosides has been reported.[269]

Several simple derivatives of 3-deoxy-D-*xylo*-hexose dialkyldithioacetals have been prepared and a route to 3-deoxy-2,6-di-*O*-methyl-D-*xylo*-hexose was described starting from the 4,5-*O*-isopropylidene acetal of the dimethylacetal.[439] In connection with immunochemical studies the *p*-nitrophenyl glycosides of 3-deoxy-D-*arabino*-, *ribo*-, and *xylo*-hexose were synthesised.[100] Extensive work has been carried out on 3-deoxyulosonic acids (see p. 136). The preparations of 4-deoxy-D-*ribo*-hexose and its D-*lyxo*-isomer have been described (Scheme 63). The oxidation proceeded as illustrated when



Scheme 63

carried out with chromic oxide in acetic acid, but in pyridine both ketones (264) and (265) were obtained. Isomerisation of (264) to (265) was effected with pyridine–DMF.[440] Methyl 4-deoxy-α-D-*xylo*-hexopyranoside was prepared as shown in Scheme 64.[441] Some racemic 4-deoxy-aldopyranoside derivatives and 4-deoxy-hexuronic acids are noted on p. 117.



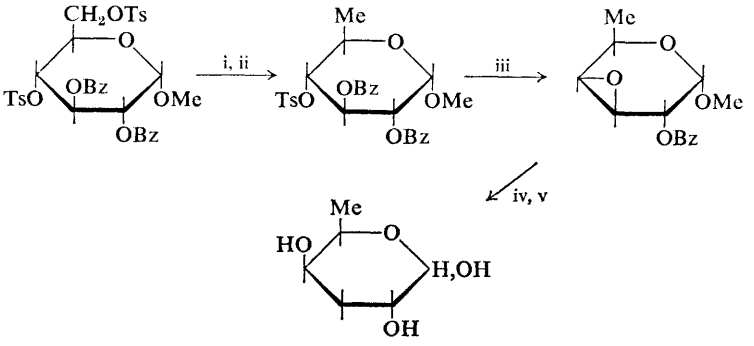
Reagents: i, NaI, DMF; ii, H_2–Pd; iii, MeONa, MeOH

Scheme 64

[438] J. Hüttermann and A. Müller, *Z. Naturforsch.*, 1969, **24b**, 463.
[439] H. Zinner and G. Wulf, *Chem. Ber.*, 1969, **102**, 180.
[440] M. Černý, J. Stanek jun., and J. Pacák, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1750.
[441] G. Siewert and O. Westphal, *Annalen.*, 1968, **720**, 161.

Full details have been published[441a] of the products from the u.v. irradiation of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-D-galactose (see vol. 2, p. 123). The first 8-deoxyoctose is noted on p. 153.

Several reports have appeared on dideoxyhexose derivatives, all having a 6-deoxy group. The structural analysis of olivomose, contained in all olivomycins, showed it to be 2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexose, and olivose and oliose are shown to be 2,6-dideoxy-D-*arabino*- and *lyxo*-hexose, respectively.[442] A new synthesis of 3,6-dideoxy-D-*xylo*-hexose (abequose) has been described (Scheme 65).[443] D-Chalcose (lankavose), a constituent of



Reagents: i, NaI, MeCOEt; ii, H_2–Pd; iii, MeONa, MeOH; iv, LAH; v, H^+

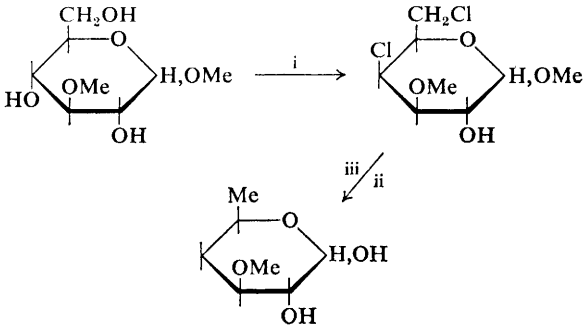
Scheme 65

several antibiotics and shown to be 4,6-dideoxy-3-*O*-methyl-D-*xylo*-hexose, has been prepared according to Scheme 66.[444] Other workers have approached the synthesis of the unmethylated sugar by use of the 6-*O*-toluene-*p*-sulphonyl analogue of the starting material for Scheme 64.[441]

Attempts to repeat the synthesis of methyl 4,6-dideoxy-α-L-*lyxo*-hexoside by the reaction of triphenyl phosphite methiodide with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (see vol. 1, p. 122) were not successful. The main product was compound (266) which afforded a 5,6-dideoxy compound on hydrogenolysis.[307] 4,6-Dideoxy-L-*ribo*-hexose has been prepared by reduction of the corresponding lactone.[445]

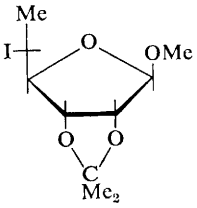
In connection with immunochemical studies, the following deoxy and dideoxy derivatives of *p*-aminophenyl α-D-galactopyranoside have been prepared: 2-, 4-, 6-, 2,6-, and 4,6-.[101] The trideoxy-hexose derivative methyl α-D-amicetoside has been prepared by standard procedures as

[441a] W. W. Binkley and R. W. Binkley, *Carbohydrate Res.*, 1969, **11**, 1.
[442] Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, and G. Yu. Pek, *Khim. prirod. Soedinenii.*, 1969, **103**, 109.
[443] G. Siewert and O. Westphal, *Annalen*, 1968, **720**, 171.
[444] B. T. Lawton, D. J. Ward, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 2899.
[445] J. Němec and J. Jarý, *Coll. Czech. Chem. Comm.*, 1969, **34**, 843.



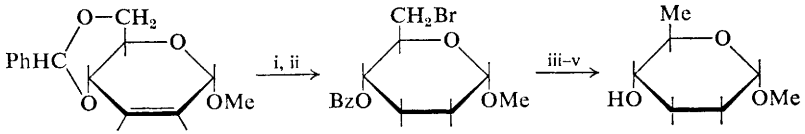
Reagents: i, SO_2Cl_2; ii, H_2–Ni; iii, H^+

Scheme 66



(266)

shown in Scheme 67.[446] A preliminary communication has reported the formation of 2,3,6-trideoxy-sugars, in which 6-deoxy derivatives with a 2,3-*cis*-diol grouping were converted into olefins by the Corey–Winter procedure, and the unsaturated products then hydrogenated.[447]



Reagents: i, H_2–Pd; ii, NBS–CCl_4; iii, KI–DMF; iv, MeOH, MeONa; v, H_2–Pd

Scheme 67

[446] E. L. Albano and D. Horton, *J. Org. Chem.*, 1969, **34**, 3519.
[447] A. H. Haines, *Carbohydrate Res.*, 1969, **10**, 466.

14
Unsaturated Derivatives

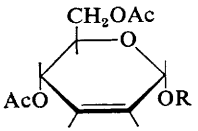
The study of unsaturated sugar derivatives continues to attract wide attention, and a large number of papers making substantial contributions to the understanding of the reactions of these compounds have appeared. A review on recent aspects has been published.[447a]

Glycals

Most recent studies on the addition of halogens and related compounds to glycals have been referred to in Chapter 7. In the course of that work, tri-*O*-acetyl-2-chloro-D-glucal was prepared and its addition to chlorine was examined.[286] Di-*O*-benzoyl-D-arabinal and -D-xylal have been obtained as new, crystalline glycal derivatives by benzoylation of the parent substances prepared from their acetates; they underwent mainly addition reactions on treatment with hydrogen bromide and hydrogen chloride in benzene.[288] Full details of the synthesis of 4,6-di-*O*-acetyl-3-bromo-2,3-dideoxy-α-D-*arabino*-hexopyranose, by treatment of tri-*O*-acetyl-D-glucal with hydrogen bromide in acetic acid have been given (*cf.* vol. 1, p. 126).[448]

D-Galactal has been found to be a powerful inhibitor of various β-D-galactopyranosidases.[449]

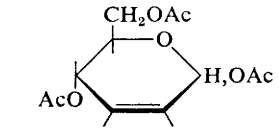
The reaction whereby tri-*O*-acetyl-D-glucal can be converted into alkyl 4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranosides (vol. 2, p. 15) has been described in detail. On treatment with equimolar proportions of alcohols in benzene in the presence of boron trifluoride the glycal ester reacted completely to give glycosides with the α-anomers predominant (*ca.* 90%); the ethyl compound (267), for example, was readily obtainable in high yield by this method. Complex alcohols were also used, as was exemplified by the preparation of, for example, the sterol compound (268) and the disaccharide derivative (269). When the reactions were carried out



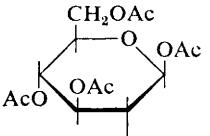
(267) R = Et
(268) R = cholesteryl
(269) R = 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl

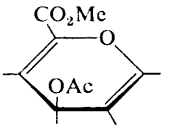
[447a] R. J. Ferrier, *Adv. Carbohydrate Chem.*, 1969, **24**, 199.
[448] T. Maki and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1968, **16**, 2242.
[449] Y. C. Lee, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 161.

with simple aliphatic acetals instead of alcohols the same products were obtained, rather than branched-chain compounds, which might have arisen by addition of the acetals to the vinyl ether double bonds.[450] Epoxidation and hydroxylation studies were then carried out on the 2,3-unsaturated glycosides produced by this method.[451] In control experiments performed during the course of the above-mentioned unsaturated glycoside synthesis, it was observed that on treatment with low concentrations of boron trifluoride, tri-*O*-acetyl-D-glucal isomerised to give (270) and that, on further reaction, the product dimerised to give C—C-linked compounds, from which (271) was obtained crystalline.[452]

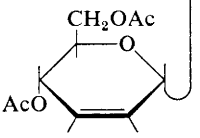


(270)

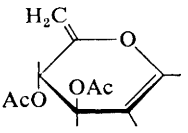




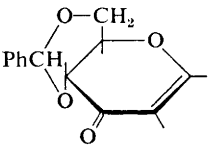
(272)



(271)



(273)

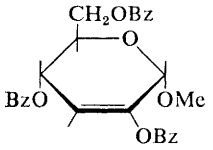


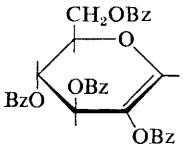
(274)

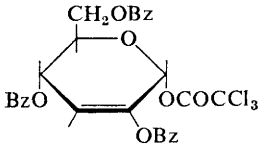
Dienes derived from glycal structures have been briefly studied; compounds having parallel bonds, such as (272), were unstable and polymerised readily, whereas dienes with the bond orientation of (273) were quite stable and readily purified.[453] The enone (274) was produced on u.v. irradiation of methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-*ribo*-hexopyranosid-3-ulose in benzene,[454] and the same compound resulted from oxidation of 4,6-*O*-benzylidene-D-allal by chromium trioxide in pyridine.[455]

Several reports have appeared on glycal derivatives which have substituents on the double bonds. It has been demonstrated that 2-hydroxyglycal esters react with alcohols in the presence of Lewis acids in the same way as do glycal esters. Compound (275), for example, was readily synthesised in this way from (276). Alternatively, crystalline β-glucosides of this series were synthesised by the uncatalysed alcoholysis of the trichloroacetate (277) which can be prepared directly from the hydroxyglycal

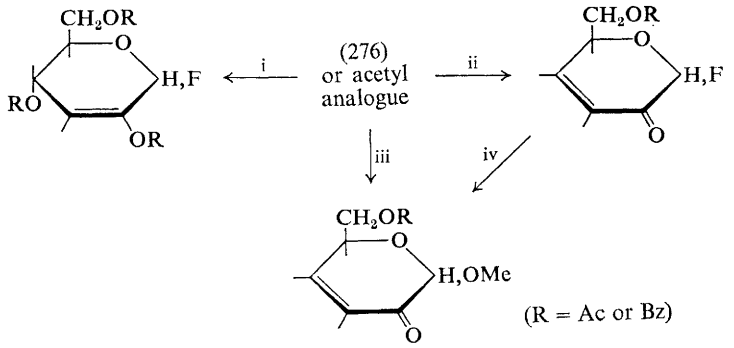
[450] R. J. Ferrier and N. Prasad, *J. Chem. Soc.* (*C*), 1969, 570.
[451] R. J. Ferrier and N. Prasad, *J. Chem. Soc.* (*C*), 1969, 575.
[452] R. J. Ferrier and N. Prasad, *J. Chem. Soc.* (*C*), 1969, 581.
[453] J. Kiss, *Carbohydrate Res.*, 1969, **11**, 579.
[454] P. M. Collins and P. Gupta, *Chem. Comm.*, 1969, 90.
[455] P. M. Collins, *Carbohydrate Res.*, 1969, **11**, 125.


(275)


(276)


(277)

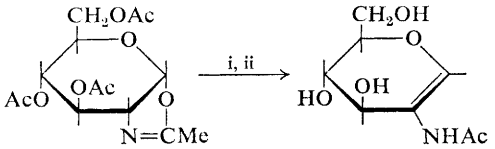
ester (276).[456] Related results obtained on the reaction of various fluorinated reagents with (276) and its acetyl analogue are summarised in Scheme 68.[457]



Reagents: i, anhydrous HF, −70°; ii, anhydrous HF, −30°; iii, MeOH, BF_3; iv, MeOH

Scheme 68

2-Acetamido-D-glucal (278) was first encountered by Pravdic and Fletcher during work on the reaction of 2-acetamido-2-deoxy-D-mannose with isopropenyl acetate in the presence of toluene-*p*-sulphonic acid (vol. 1, p. 93). Other, more efficient approaches to the synthesis of this potentially valuable material have now been reported. Heating the acetylated α-D-*gluco*-oxazoline (279) in tetramethylurea, containing a trace of the same catalyst, caused isomerisation, and the formation of the triacetate of (278) in 52% yield as shown in Scheme 69. The β-D-*manno*-oxazoline acetate

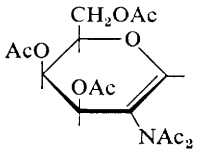

(279) (278)

Reagents: i, heat, H^+; ii, MeOH, MeONa

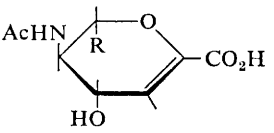
Scheme 69

[456] R. J. Ferrier, N. Prasad, and G. H. Sankey, *J. Chem. Soc.* (*C*), 1969, 587.
[457] K. Bock and C. Pedersen, *Tetrahedron Letters*, 1969, 2983.

gave the same product, and, not unexpectedly, the same type of reaction can be used to obtain 2-acetamido-D-galactal.[348, 458] Treatment of 2-acetamido-2-deoxy-D-galactose with boiling isopropenyl acetate containing a trace of toluene-*p*-sulphonic acid gave a product, originally formulated as a 2,3-unsaturated pyranose derivative (vol. 1, p. 95). Hydrogenation of its de-*O*-acetylated analogue gave 2-acetamido-1,5-anhydro-2-deoxy-D-talitol and this, taken with detailed n.m.r. results, has established that the initial compound was (280).[348]



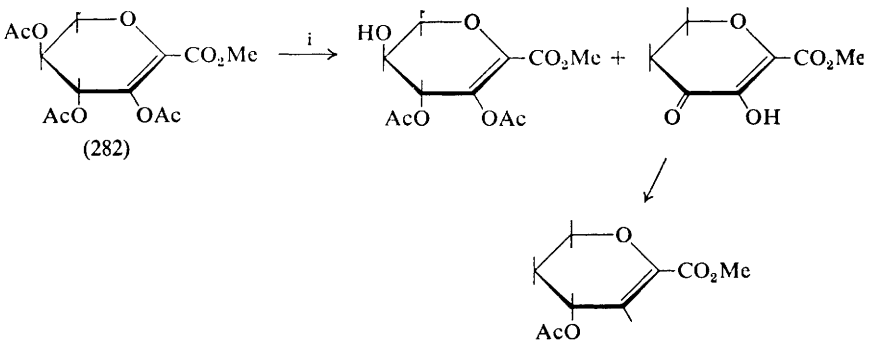
(280)



(281)

The syntheses of 3-acetamido-3,6-dideoxy-1,2-*O*-isopropylidene-α-D-gluco-5-enose, and its *allo* isomer, have been described.[458a]

Carbon-substituted glycals have also been reported. Treatment of *N*-acetyl-4,7,8,9-tetra-*O*-acetyl-2-chloro-2-deoxy-neuraminic acid with bases, or heating the *N*-acetyl-2,4,7,8,9-penta-*O*-acetate in dioxan and subsequent deacetylation, gave the acid (281). Other related unsaturated compounds were also described.[459] Another such aldonic acid derivative (282) has been examined and its reactions in acid were briefly reported (Scheme 70).[460]



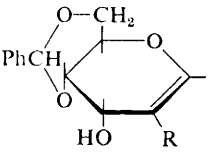
(282)

Reagents: i, AcOH, H_2O, 180°

Scheme 70

[458] W. L. Salo and H. G. Fletcher jun., *J. Org. Chem.*, 1969, **34**, 3189.
[458a] H. Ohrui and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1371.
[459] P. Meindl and H. Tuppy, *Monatsh.*, 1969, **100**, 1295.
[460] K. Goshima and K. Tokuyama, *Tetrahedron Letters*, 1969, 2383.

Reaction of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside with pentachlorophenyl-lithium gave mainly a branched-chain product but



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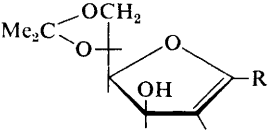
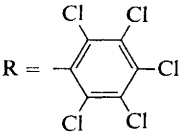


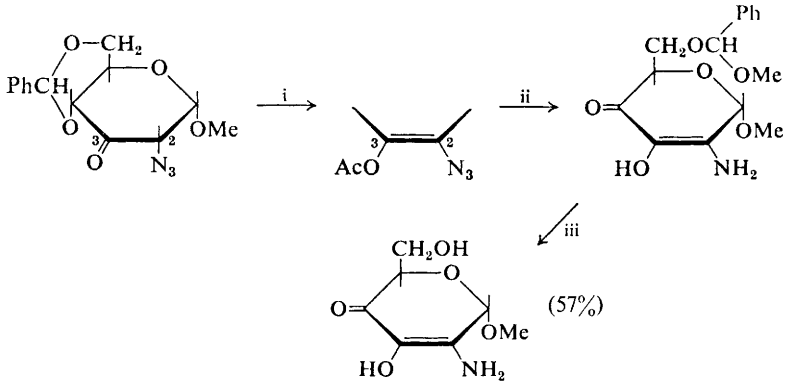
(284)



also (283) as a minor product. The same reagent with 2,3:5,6-di-*O*-isopropylidene-α-D-mannosyl chloride gave (284).[460a]

Other Unsaturated Compounds

It has been found that DMSO-based oxidations of sugar acetates having one free hydroxy group generally gave enones formed by eliminations β- to the newly generated carbonyl function; kojic acid derivatives and other dienones have also been obtained by this method (p. 167). Further enones were encountered as outlined in Scheme 71.[461] It had earlier been shown



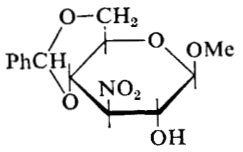
Reagents: i, Ac_2O, pyr; ii, NaOMe, MeOH; iii, H^+

Scheme 71

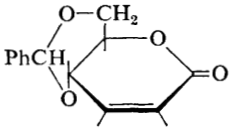
that treatment of compounds such as (285) with alcohols or amines in refluxing toluene in the presence of basic alumina caused the replacement

[460a] J. B. Lee and B. Scanlon, *Chem. Comm.*, 1969, 955.
[461] W. Meyer zu Reckendorf, *Naturwiss.*, 1969, **56**, 328.

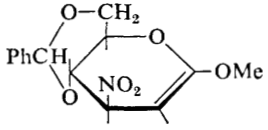
of the hydroxy-groups by alkoxy or amino functions; the reactions proceeded by way of nitro-olefins. It has now been established that such treatment in the absence of the nucleophilic species results in unsaturated lactones, *e.g.* (286), by rearrangements of the nitro-olefins, to give intermediates such as (287), followed by hydrolysis to lactones and β-eliminations of nitrous acid.[462]



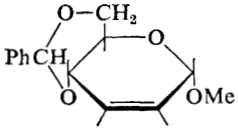
(285)



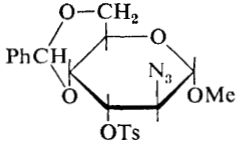
(286)



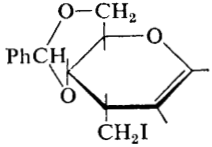
(287)



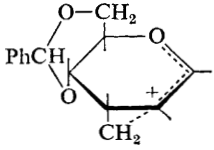
(288)



(289)



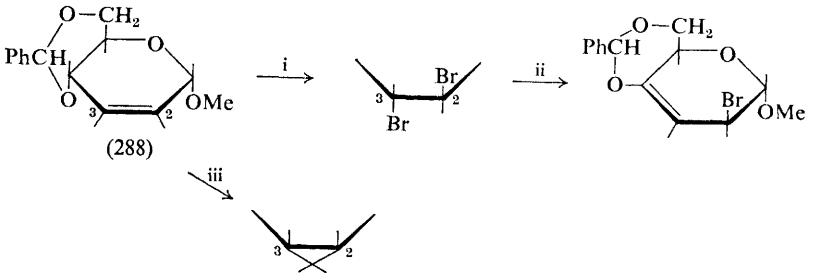
(290)



(291)

A modification of the potassium iodide, zinc, DMF procedure applied to vicinal disulphonylated carbohydrates has used zinc–copper couples and has provided a means for obtaining (288) in quantity, and analogues with acyl groups at C-4 and C-6 in place of the acetal ring. Elimination from methyl 4,6-di-*O*-acetyl (or benzoyl) -2,3-di-*O*-methanesulphonyl-α-D-glucopyranoside was accompanied by partial deacylations, which occur preferentially at C-4 without inversion of configuration.[463] Reaction of (289) with an excess of hydrazine hydrate affords a further, convenient synthesis of (288).[464] Some reactions of (288) are outlined in Scheme 72.[465] This compound has also been utilised in a synthesis of the 2,3,6-trideoxyhexose amicetose.[446] Other workers have noted a by-product (290),

[462] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1969, **47**, 2811.
[463] B. Fraser-Reid and B. Boctor, *Canad. J. Chem.*, 1969, **47**, 393.
[464] R. D. Guthrie, R. D. Wells, and G. J. Williams, *Carbohydrate Res.*, 1969, **10**, 172.
[465] E. L. Albano, D. Horton, and J. H. Lauterbach, *Carbohydrate Res.*, 1969, **9**, 149.

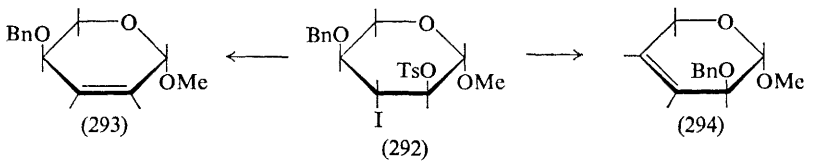


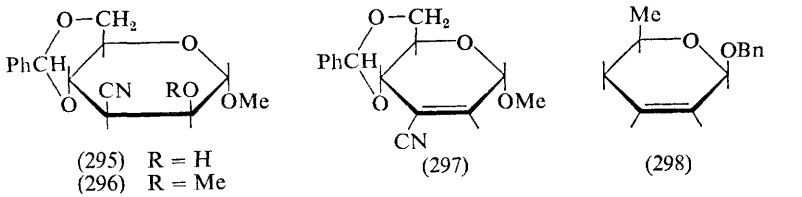
Reagents: i, Br_2, MeOH, AgOAc, $BaCO_3$; ii, Bu^tOK; iii, CH_2I_2, Zn, Cu

Scheme 72

formed together with the *allo*-cyclopropyl adduct, on treatment of (288) with the Simmons–Smith reagent; the iodide atom in (290) was highly susceptible to nucleophilic displacement. These observations were rationalised by invoking the resonance stabilised ion (291) as an intermediate.[466]

An interesting rearrangement has been observed during an apparently simple elimination reaction. Thus, treatment of (292) with sodium iodide in acetone gave the expected olefin (293) only at reduced temperatures; at room temperature (294) was also produced. A mechanism for the rearrangement was proposed. The synthesis of methyl 4-*O*-benzyl-2,3-dideoxy-6-*O*-trityl-α-D-*erythro*-hex-2-enopyranoside was also reported.[467]



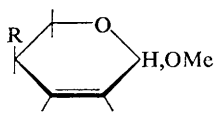


Attempted brosylation of (295) yielded (297), which with sodium methoxide in methanol added solvent to afford (296). The reactivity of

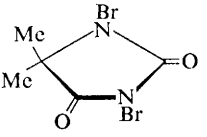
[466] B. Fraser-Reid and B. Radatus, *Canad. J. Chem.*, 1969, **47**, 4095.
[467] S. Dimitrijevich and N. F. Taylor, *Carbohydrate Res.*, 1969, **11**, 531.

compound (297) was compared with that of the known nitro-olefinic analogue.[468] Further work has been reported on 2,3-unsaturated 3-nitro compounds.[395]

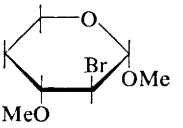
A series of papers has appeared on the chemistry of racemic 2,3-unsaturated pyranosides synthesised from non-carbohydrate precursors. Compounds with hydrogen,[469] methyl (*e.g.* 298),[469, 470] hydroxymethyl,[471] carboethoxy,[470, 472, 473] aminomethyl,[478] and carboxamido [472] groups at C-5 were prepared by a Russian group, and their epoxidation reactions and the aminolysis of the products were examined. In related studies, Canadian workers investigated the reactions of 2,4-dimethoxy-tetrahydropyran and obtained (299) by heating with phosphoric oxide. Treatment with (301) in ether–methanol gave (302) and (303) in the ratio 2 : 1.[474]



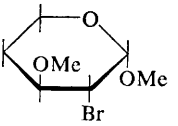
(299) R = H
(300) R = OH



(301)



(302)



(303)

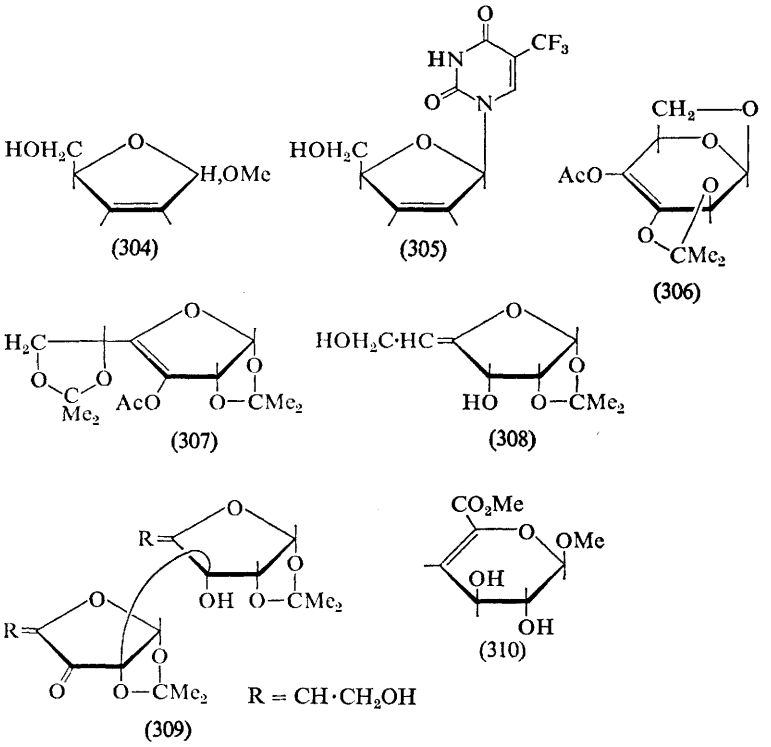
Enones obtained by oxidation of alkyl 2,3-dideoxy-6-substituted-α-D-*erythro*-hex-2-enopyranosides are mentioned in Chapter 16.

In the furanoid series, the pure anomeric forms of (304) have been synthesised from the corresponding 2,3-epoxides, and the n.m.r. spectra of the products and their esters were examined in detail, and the rings found to be essentially planar. Similarly, the anomeric forms of (300) were prepared and studied. Valuable discussions of many aspects of the optical activities and n.m.r. spectra of these compounds are provided.[475]

[468] B. E. Davison and R. D. Guthrie, *Carbohydrate Res.*, 1969, **9**, 254.
[469] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei. Khim.*, 1969, **39**, 681.
[470] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei. Khim.*, 1969, **39**, 701.
[471] V. B. Mochalin, Yu. N. Porshnev, G. I. Samokhvalov, and M. Ts. Yanotovskii, *Zhur. obshchei. Khim.*, 1969, **39**, 116.
[472] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei. Khim.*, 1969, **39**, 420.
[473] V. B. Mochalin, Yu. N. Porshnev, and G. I. Samokhvalov, *Zhur. obshchei. Khim.*, 1969, **39**, 109.
[474] M. J. Baldwin and R. K. Brown, *Canad. J. Chem.*, 1969, **47**, 3099.
[475] R. U. Lemieux, K. A. Watanabe, and A. A. Pavia, *Canad. J. Chem.*, 1969, **47**, 4413.

The unsaturated nucleoside (305) has been obtained from the 2′-deoxy-3′-*O*-methanesulphonyl-5′-*O*-trityl-analogue, and the corresponding D-ribofuranosyl nucleoside was also prepared by standard procedures. Biological tests were carried out on the products.[476]

Elimination to give a 3,4-unsaturated pyranoid compound has been noted when methyl 4,6-*O*-benzylidene-3-chloro-3-deoxy-β-D-allopyranoside was treated with nucleophiles (p. 87), and compound (306), with the unusual substituted enediol structure, was formed from the corresponding 4-ulose derivative (p. 130).

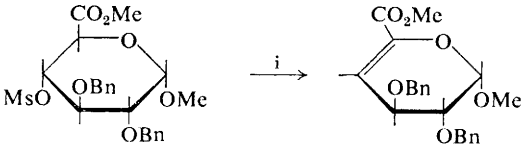


Reduction of the 3,4-unsaturated compound (307) with sodium borohydride gave 1,2:5,6-di-*O*-isopropylidene-α-D-gulose as expected, but, in addition, a mixture of *cis*- and *trans*-olefins (308), presumably formed by way of the corresponding α,β-unsaturated ketones which were obtainable by treatment of (307) with mild alkali. During this reaction, however, the aldol dimeric products (309) were also formed. The 1,2-phenyloxazoline corresponding to (307) gave olefinic analogues of (308) with sodium borohydride.[477]

[476] T. A. Khwaja and C. Heidellberger, *J. Medicin. Chem.*, 1969, **12**, 543.
[477] W. Meyer zu Reckendorf and J. C. Jochims, *Chem. Ber.*, 1969, **102**, 4199.

The formation of (310) from the galacturonic acid methyl ester methyl glycoside has been examined in detail, and the catalytic reduction to the C-5 epimeric 4-deoxy-D-*xylo*- and L-*arabino*-esters in the ratio 14 : 86, discussed. From the α-anomer of (310) the corresponding hydrogenation products were formed in the ratio 95 : 5. These ratios were considered in relationship to stereochemical factors.[478]

Related unsaturated compounds can be prepared by eliminations β- to hexuronic ester groups as is illustrated in Scheme 73.[479]

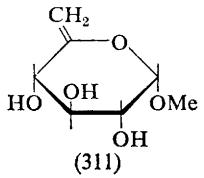


Reagents: i, MeOH, MeONa

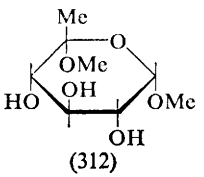
Scheme 73

A series of 4,5- and 5,6-unsaturated derivatives of alkyl 2-amino-2-deoxy-hexopyranosides were prepared by β-eliminations from 4-sulphonylated hexuronates followed by reductions of the uronate esters, and by eliminations from 2-amino-2,6-dideoxy-6-iodo-glycosides, respectively. The 5,6-unsaturated compounds were the more readily hydrolysed with acid (to give 6-deoxy-5-ulose derivatives).[480]

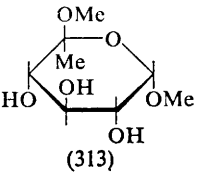
A 5,6-unsaturated pyranoid compound was also obtained in addition to the 6-deoxy analogue, when 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was irradiated with u.v. light in t-butyl alcohol.[441] Also n this series, compound (311) has been treated with methanol in the presence of acid, and the epimeric compounds (312) and (313) were obtained.[118]



(311)



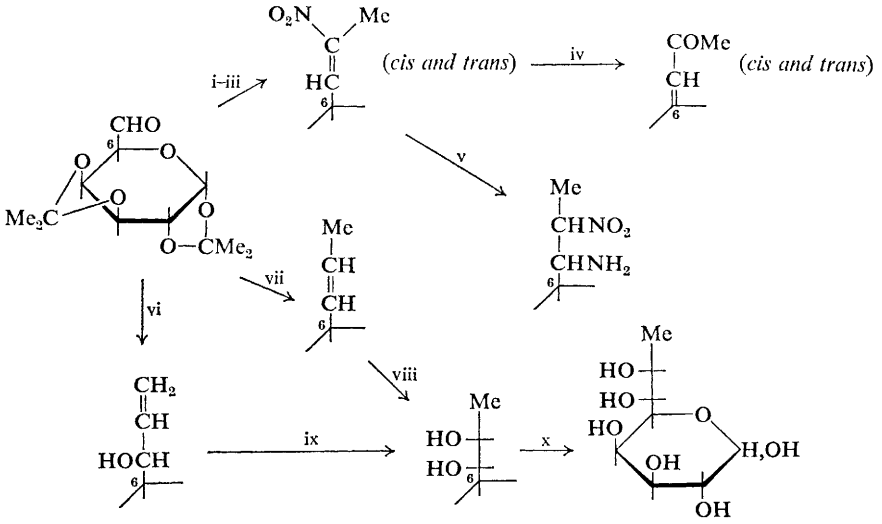
(312)



(313)

Various additions to the dialdose (314) have resulted in octose derivatives having unsaturation in the acyclic parts of the molecules. Their syntheses and reactions are outlined in Scheme 74.[481, 482]

[478] H. W. H. Schmidt and H. Neukom, *Carbohydrate Res.*, 1969, **10**, 361.
[479] J. Kiss, *Carbohydrate Res.*, 1969, **10**, 328.
[480] J. Kiss and F. Burkhardt, *Helv. Chim. Acta*, 1969, **52**, 2622.
[481] G. B. Howarth, D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 75, 81.
[482] D. G. Lance, W. A. Szarek, J. K. N. Jones, and G. B. Howarth, *Canad. J. Chem.*, 1969, **47**, 2871.



Reagents: i, $EtNO_2$, MeOH, MeONa; ii, Ac_2O, H_2SO_4; iii, Et_3N; iv, *hν* (254 nm); v, NH_3; vi, CH_2=CHMgBr; vii, MeCH=PPh_3; viii, $KMnO_4$; ix, $Hg(OAc)_2$, H_2O; x, H^+

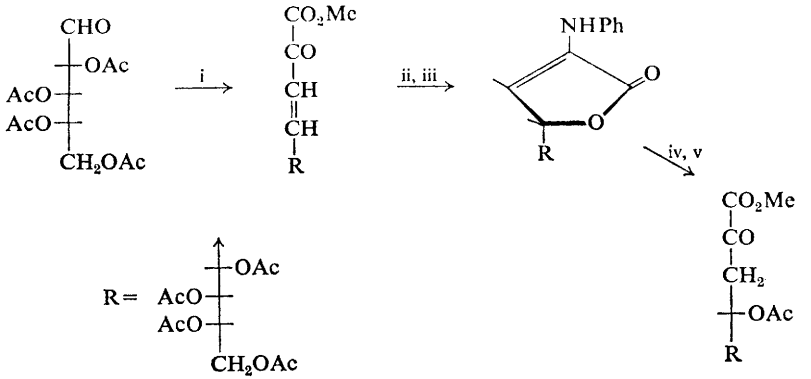
Scheme 74

Russian workers, in particular, have continued the investigation of the reaction of Wittig reagents with *aldehydo*-sugars, and reference has already been made to the development of a new synthesis of 2-deoxy-aldoses which used alkoxy- or alkylthio-methylenetriphenylphosphoranes. Deoxy-ketose derivatives have been synthesised by use of pyruvylidene analogues[482a] and deoxyketoaldonic acids as shown in Scheme 75.[483] Other Russian work has described the preparation of new unsaturated Wittig adducts from *aldehydo*-acetal derivatives[484] and from partially methylated aldoses.[485] A new type of derivative in this series, for example (315), has been prepared using ylides derived from maleimide and *N*-phenyl-maleimide.[486]

Treatment of 3,4,5,6,7-penta-*O*-acetyl-1-deoxy-1-diazo-D-heptulose with silver oxide has been found to give *trans*-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*arabino*-hept-2-enonic acid, instead of the expected 3,4,5,6,7-penta-*O*-acetyl-2-deoxy-D-*gluco*-heptonic acid.[487]

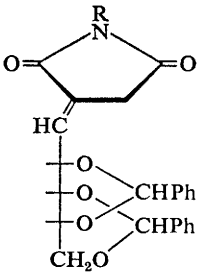
The n.m.r. spectra of acyclic nitro-olefins are referred to in Chapter 23. Various unsaturated aldonic acid derivatives are reported in Chapter 17

[482a] Yu. A. Zhdanov and V. A. Polenov, *Zhur. obshchei. Khim.*, 1969, **39**, 1121, 1124.
[483] B. A. Dmitriev, L. V. Backinowsky, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1968, 2341.
[484] Yu. A. Zhdanov and V. G. Alekseeva, *Zhur. obshchei. Khim.*, 1969, **39**, 405, 112.
[485] Yu. A. Zhdanov and V. A. Polenov, *Zhur. obshchei. Khim.*, 1969, **39**, 119.
[486] R. E. Harman, G. Wellman, and S. K. Gupta, *Carbohydrate Res.*, 1969, **11**, 574.
[487] D. Charon, *Carbohydrate Res.*, 1969, **11**, 447.



Reagents: i, $Ph_3PCH \cdot COCO_2Me$; ii, CF_3CO_2H; iii, $PhNH_2 \cdot HCl$; iv, MeOH, H^+; v, Ac_2O, pyr

Scheme 75



(R = H or Ph)

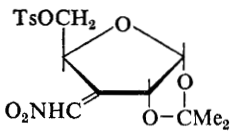
(315)

and olefins formed by Wittig reactions in Chapter 16. An exocyclic olefin formed from di-*O*-isopropylidene-D-glucofuranose has been used to synthesise branched-chain nitrosugars.[394]

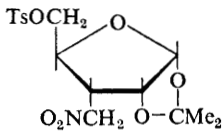
15
Branched-chain Sugars

Although little has been reported on the isolation of new naturally occurring branched-chain sugars there has been considerable interest in the synthesis of compounds of this type. Many nucleosides containing branched-chain sugars have been prepared (see also Chapter 21).

One of the most interesting reports of branched-chain sugar synthesis has concerned the reaction of ulose derivatives with nitromethane.[277] Thus, reaction of (191) with nitromethane in the presence of sodium hydride afforded (67). The amino-sugar formed on reduction of (67) underwent an intramolecular displacement reaction to yield an epimino derivative.[277]



(316)



(317)

Also, (67) was converted into (316), and then into the branched-chain deoxy-sugar (317).

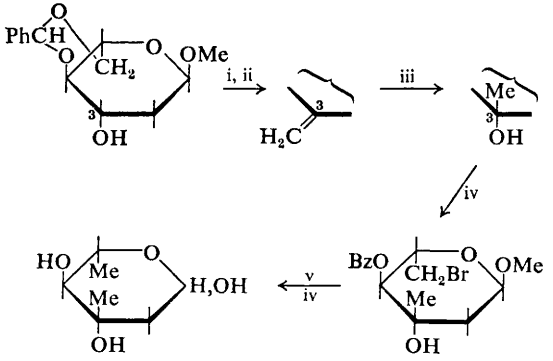
Compounds with an $R^1—C—OR^2$ Branch

Much of the work on sugars of this type has been connected with naturally occurring products. The structure of olivomycose, the sugar component of olivomycin, has been confirmed [488] by the synthesis outlined in Scheme 76.

The di-*O*-acetate (318) of another naturally occurring sugar, arcanose, has been fused with 6-chloropurine in the presence of toluene-*p*-sulphonic acid to give (319), the first synthetic nucleoside containing a naturally occurring branched-chain sugar.[489] Interestingly, treatment of methyl arcanoside 4-acetate with dichloromethane saturated with hydrogen chloride yielded an unsaturated compound having an n.m.r. spectrum consistent with (320) rather than the expected glycosyl halide.

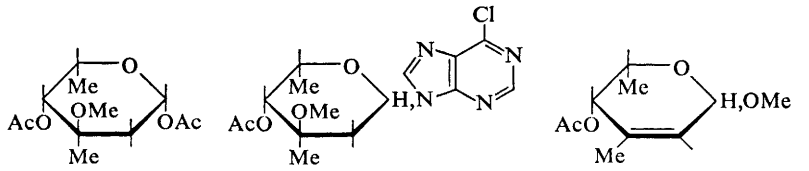
The assignment of the structure of hamamelitannin as the 1,5-di-*O*-galloyl derivative of 2-*C*-hydroxymethyl-D-ribofuranose has been confirmed by synthesis.[234] More syntheses of apiose derivatives have been

[488] E. H. Williams, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 4467.
[489] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *J. Org. Chem.*, 1969, **34**, 476.



Reagents: i, RuO_2, KIO_4; ii, $Ph_3P{=}CH_2$; iii, $Hg(OAc)_2$, $NaBH_4$; iv, NBS; v, LAH, vi, H^+

Scheme 76

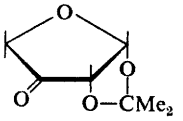


(318) (319) (320)

recorded: reaction of 1,2-*O*-isopropylidene-α-D-*glycero*-tetros-3-ulose (321) with diazomethane yielded an epimeric mixture of epoxides, from which 1,2-*O*-isopropylidene-3,1′-anhydro-α-D-apio-D-furanose (322) was isolated. Reaction of (322) with aqueous methanolic sodium methoxide afforded the isopropylidene derivative (323), which was hydrolysed to D-apiose.[490] The isolation of a second isomer (324) of di-*O*-isopropylidene-D-apiose has been reported.[491] 3-Deoxy-1,2-*O*-isopropylidene-α-D-apio-L-furanose (325) has been prepared by the stereoselective hydroboration of 3-deoxy-1,2-*O*-isopropylidene-3-*C*-methylene-D-*glycero*-tetrose (326). 3-Deoxy-D-apiose may be distinguished from 3-deoxy-*erythro*-pentose by its colour reaction with *p*-anisidine hydrochloride.[491] A sensitive and specific method of determining D-apiose, based on the reaction with cysteine after acidic degradation, has been reported.[492] An apiose analogue with nitrogen as the ring hetero-atom has been described.[428a]

The structure of L(+)-dihydrodeoxystreptose, a 3,5-dideoxy-3-*C*-hydroxymethyl-L-xylofuranose, has been confirmed by n.m.r. spectroscopic and mass spectrometric studies of glycosidic and aldonic acid derivatives.[493]

[490] A. D. Ezekiel, W. G. Overend, and N. R. Williams, *Tetrahedron Letters*, 1969, 1635.
[491] D. H. Ball, F. A. Carey, I. L. Klundt, and L. Long jun., *Carbohydrate Res.*, 1969, **10**, 121.
[492] H. Sandermann jun., *Phytochemistry*, 1969, **8**, 1571.
[493] H. Ikeda, K. Murofushi, and H. Ikada, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1968, **62**, 91 (*Chem. Abs.*, 1969, **70**, 4476c).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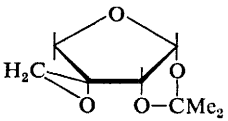
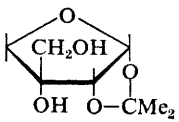

(322)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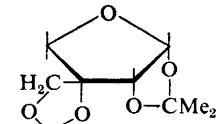
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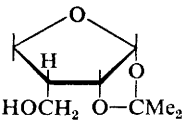
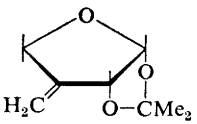
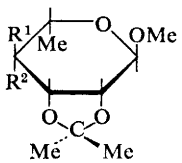
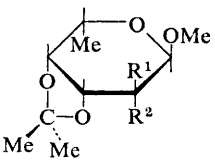

(325)


(326)


(327)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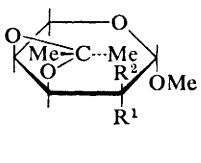
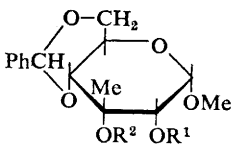

(329)

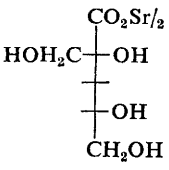
(R^1 and R^2 are variously H, or OH, or CH_2NO_2, or CH_3)

It has been shown that in the n.m.r. spectra of branched-chain methyl isopropylidene-glycopyranosides of the type shown, (327), (328), (329), the *endo* methyl is at higher field when *cis* to the branched substituent than when *trans* to it.[493a]

The formation of four products (331)—(334) from the reaction between the toluene-*p*-sulphonyl derivative (330) and sodium methoxide in dry



	R^1	R^2
(330)	Ts	H
(331)	H	H
(332)	H	Me
(333)	Me	Me
(334)	Me	H


(335)

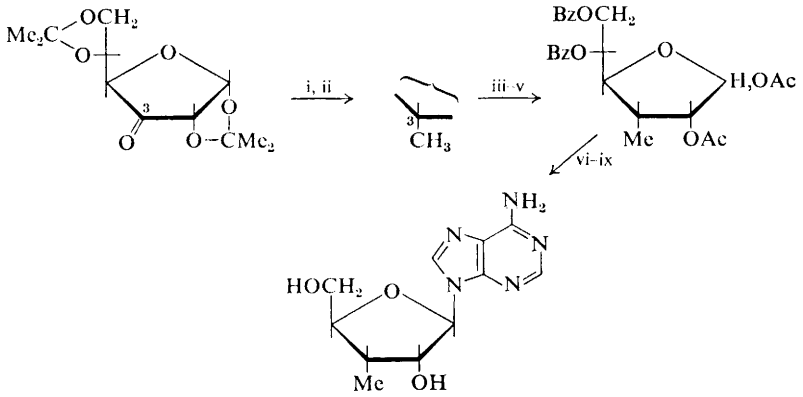
methyl sulphoxide has been rationalised.[494] Compound (332) has been converted into 6-deoxy-3-*C*-methyl-3-*O*-methyl-D-allose (2-hydroxy-D-cladinose).

[493a] R. D. King and W. G. Overend, *Carbohydrate Res.*, 1969, **9**, 423.
[494] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1969, **11**, 257.

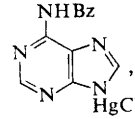
The strontium salt of the isosaccharinic acid obtained by the reaction of 4-*O*-substituted-D-glucoses with strontium hydroxide has been shown by *X*-ray studies to have the D-*erythro* structure (335).[495]

Compounds with an R—C—H Branch

Considerable use has been made of reactions of keto-sugars with Wittig reagents as a means for introducing a R—C—H branch in sugar derivatives. Thus, 3-deoxy-3-methyl-adenosine and some related nucleoside derivatives related to cordycepin were synthesised by the standard procedures outlined in Scheme 77.[496] By similar procedures (336), which was obtained by



Reagents: i, $Ph_3P{=}CH_2$; ii, H_2–Pd; iii, H^+; iv, BzCl, pyr; v, Ac_2O, AcOH, H^+;

vi,  , $TiCl_4$; vii, NaOMe, MeOH; viii, IO^-; ix, $NaBH_4$

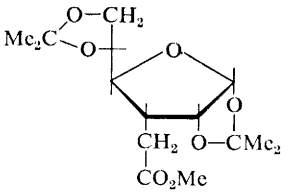
Scheme 77

application of a Wittig reaction, has been converted into nucleoside derivatives.[497]

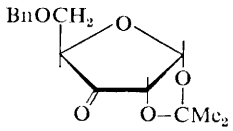
Branched-chain amino-sugars having a terminal primary amino function have been prepared from carbohydrate ketones by a modified Wittig reaction.[498] Thus, reaction of (337) with $(EtO)_2P(O)CH_2CN$ and subsequent reduction afforded (338) which was smoothly converted into (339) by sequential LAH reduction and acetylation.

Another method for the formation of nitrogen-containing branched-chain sugars involved the addition of nitryl iodide to Wittig products.[394]

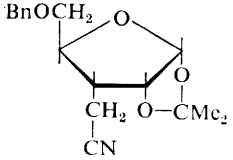
[495] P.-E. Werner, R. Norrestam, and O. Rönnquist, *Acta Cryst.*, 1969, **25***B*, 714.
[496] A. Rosenthal and M. Sprinzl, *Canad. J. Chem.*, 1969, **47**, 3941.
[497] A. Rosenthal and L. (Benzing) Nguyen, *J. Org. Chem.*, 1969, **34**, 1029.
[498] A. Rosenthal and D. A. Baker, *Tetrahedron Letters*, 1969, 397.



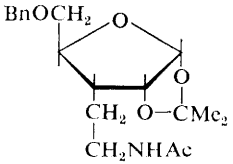
(336)



(337)

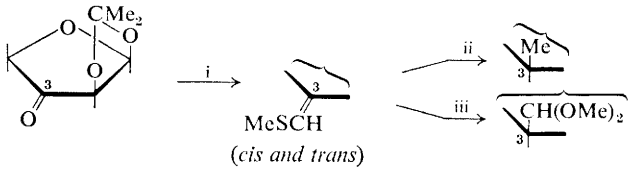


(338)



(339)

Wittig reactions between methylthiomethylenetriphenylphosphorane with various ald-3-ulofuranose derivatives have been shown to provide a useful route to branched-chain deoxy-sugars [499] (Scheme 78). Isomerisa-



Reagents: i, $Ph_3P{=}CHSMe$; ii, H_2–Ni; iii, $Hg(OAc)_2$, MeOH

Scheme 78

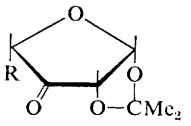
tion about C-4 occurred during the Wittig reaction with the *ribo*-isomer (340).

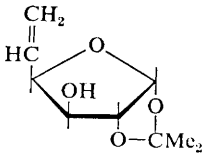
A novel method for the extension of sugar chains, or for the preparation of branched-chain sugars, has been reported. The photochemically induced addition of 1,3-dioxolan in acetone to (341) yielded (342), and similar addition of 1,3-dioxolan to (343) afforded (344).[500]

The branched-chain derivative (345) has been described.[466]

The oxo reaction has been employed [501] in the synthesis of (346) in 30% yield from (347) (*cf.* vol. 2, pp. 144—145). Compound (346) has been used in the synthesis of branched-chain nucleosides. Similarly, branched-chain nucleosides have been synthesised [502] from (348) prepared by hydroboration and suitable modification of (349).

[499] J. M. J. Tronchet, J. M. Bourgeois, R. Graf, and J. Tronchet, *Compt. rend.*, 1969, **269** *C*, 420.
[500] J. S. Jewell and W. A. Szarek, *Tetrahedron Letters*, 1969, 43.
[501] A. Rosenthal, M. Sprinzl, and H. J. Koch, *Canad. J. Chem.*, 1969, **47**, 3263.
[502] A. Rosenthal and M. Sprinzl, *Canad. J. Chem.*, 1969, **47**, 4477.





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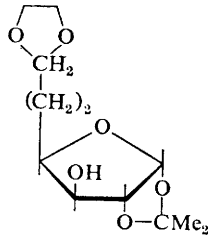
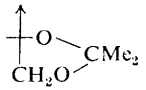


(342)

R =



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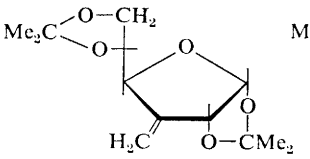

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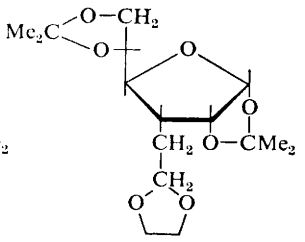

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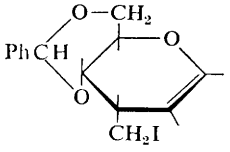

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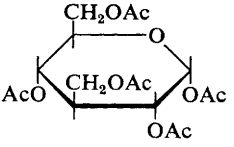

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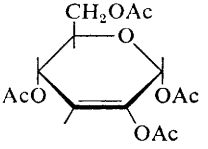

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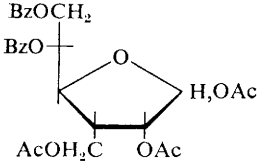

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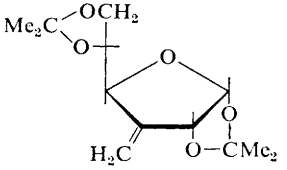
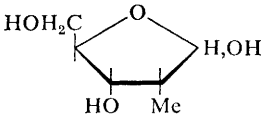
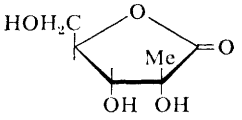


(349)

(2*R*)-2-*C*-Methyl-2-deoxy-*erythro*-D-pentose (350) has been synthesised from 2-*C*-methyl-D-ribonolactone (351).[503] The secondary hydroxy-groups in (351) were suitably protected and then the tertiary hydroxy-group



(350)



(351)

replaced by use of hydrogen bromide. Hydrogenolysis of the product afforded a mixture of (2*R*)- and (2*S*)-deoxy-2-methyl derivatives, which were separated and assigned structures on the basis of o.r.d. measurements. Di-isoamylborane reduction of the 2-deoxylactone with the 2*R* configuration and removal of protecting groups gave (350). Standard procedures were used to convert (350) into branched-chain nucleosides of thymine and adenine. Treatment of 2-*C*-methyl-D-ribose with benzyl alcohol gave primarily the β-furanoside derivative.[504]

Branched-chain sugars prepared by cyclisation of D′- or L′-methoxy diglycolaldehyde with ethyl nitroacetate have already been described.[392]

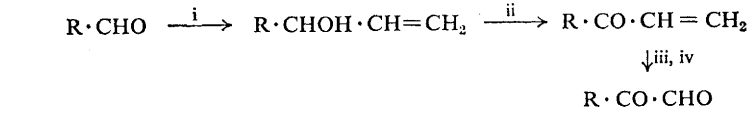
[503] J. J. K. Novák, J. Smejkal, and F. Šorm, *Tetrahedron Letters*, 1969, 1627.
[504] J. J. K. Novák and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 857.

16
Alduloses, Dialdoses, and Diuloses*

Some syntheses of free, deoxy-, amino-, and branched-chain sugars by way of keto-sugar intermediates have been reviewed.[505] D-*altro*-3-Heptulose and sedoheptulose have been isolated from the methanolic extract of the red fruit of *Coriaria japonica*.[506]

Derivatives of phenyl 2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose and of phenyl and methyl 3-deoxy-α (and β)-D-*threo*-hexopyranosid-2-ulose have been prepared by DMSO–acetic anhydride oxidation of suitably protected precursors. The stereochemical aspects of the reduction of these compounds have been examined.[507] The oxidation of D-arabinitol with a boiling methanolic solution of mercury(II) acetate gave D-*threo*-pentulose, D-*threo*-3-pentulose, and D-*erythro*-pentulose in small yields.[508] Similar oxidation of xylitol afforded DL-*threo*-pentulose and DL-*erythro*-3-pentulose.

aldehydo-Aldoses, fixed in the acyclic form by the formation of isopropylidene acetals, have been converted into aldos-2-uloses by the method shown in Scheme 79.[509]



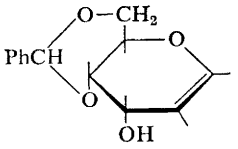
Reagents: i, CH_2=CH·MgCl; ii, MnO_2; iii, O_3; iv, H_2–Pt

Scheme 79

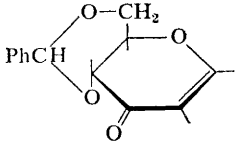
Chromium trioxide in pyridine was reported as the most satisfactory oxidant for converting (352) into (353).[455] Unsaturated compounds such as (354) were successfully oxidised to crystalline enones, for example (355), in good yield with manganese dioxide, and the alcohol (356) was converted into tosyl, benzoyl, and acetyl derivatives by standard esterifications in cold pyridine.[510] The optical rotation of (355) in aqueous acetone

[505] J. S. Brimacombe, *Angew. Chem. Internat. Edn.*, 1969, **8**, 401.
[506] T. Okuda and K. Konishi, *J. Pharm. Soc. Japan*, 1968, **88**, 1329.
[507] K. Antonakis, *Bull. Soc. chim. France*, 1969, 122.
[508] Ľ. Stankovic, K. Ľinek, and M. Federoňko, *Carbohydrate Res.*, 1969, **10**, 579.
[509] D. J. Walton, *Canad. J. Chem.*, 1969, **47**, 3483.
[510] B. Fraser-Reid, A. McLean, and E. W. Usherwood, *J. Amer. Chem. Soc.*, 1969, **91**, 5329.

* See also Chapter 22.



(352)



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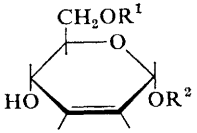

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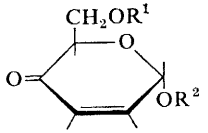
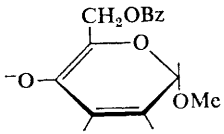


(355) R^1 = Bz, R^2 = Me
(356) R^1 = H, R^2 = Et



(357)

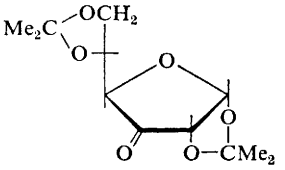
containing triethylamine changed, but on evaporation of the solvents starting material was recovered. N.m.r. spectroscopic evidence indicated that the ion (357) had been formed. Compound (356) in aqueous methanol containing triethylamine gave formaldehyde as a consequence of retroaldol cleavage. Other products could not be identified.[510] Synthesis of other enones has already been described [461, 477] (see also Chapter 22).

Radiolysis of methyl α-D-glucopyranoside afforded the 6-aldehydo-derivative (21).[132]

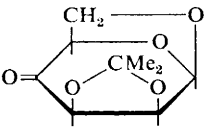
The ketone (358) has been shown to exist in aqueous organic solvents in equilibrium with its hydrate. The previously reported acetylation (vol. 1, p. 129), which gave the 3,4-enol acetate and the subsequent reduction to 1,2:5,6-di-*O*-isopropylidene-D-gulose, have been described in detail.[511] A by-product of the reduction reaction was the 3-deoxy analogue of the main product.

A novel reaction sequence has been reported [512] in which 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (359), a keto-sugar having a [3,2,1] bicyclic ring system, was converted into dimer (360) by treatment with acetic anhydride and triethylamine at room temperature. Compound (361), a 3,4-enediol acetate, was formed by heating (360) in acetic anhydride and triethylamine; for steric reasons no 4,5-enediol acetate was formed.

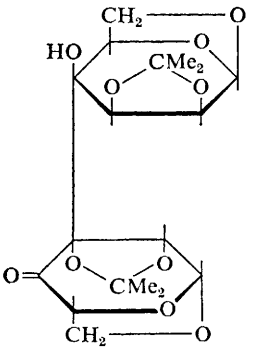
[511] W. Meyer zu Reckendorf, *Chem. Ber.*, 1969, **102**, 1071.
[512] D. Horton and E. K. Just, *Carbohydrate Res.*, 1969, **9**, 129.



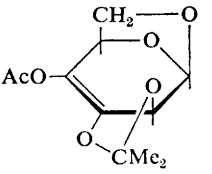
(358)



(359)

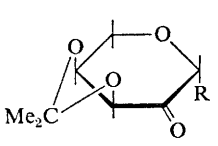


(360)

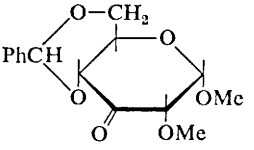


(361)

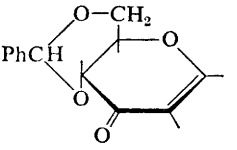
U.v. irradiation of methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentosidulose (362) in tertiary butanol gave the 1,5-anhydro compound (363).



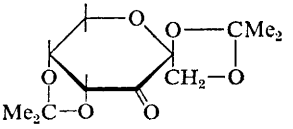
(362) R = OMe
(363) R = H



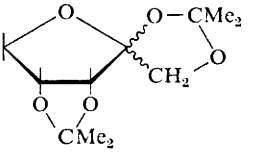
(364)



(365)



(366)

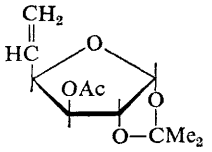


(367)

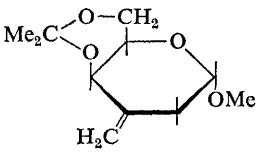
It was suggested that loss of methoxy occurred *via* the 1,2-enolic form of (362). Irradiation of methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-*ribo*-hexopyranosid-3-ulose (364) in benzene yielded four products, one of which was the α,β-unsaturated ketone (365).[454]

Irradiation of 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiulose-2,6-pyranose (366) gave the anomers of 1,2:3,4-di-*O*-isopropylidene-*erythro*-hexulose (367) in an α:β ratio of 1 : 2.[513] Only the β-isomer has been described previously. Hydrolysis of either anomer, or of the anomeric mixture, gave D-*erythro*-pentulose.

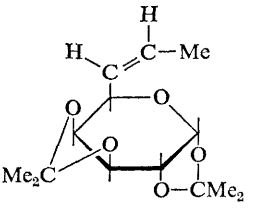
Considerable use has been made of the reaction between Wittig reagents and keto-sugars for the formation of branched-chain sugars (see Chapter 15). For example, compounds (368)—(370) have been formed by the



(368)



(369)

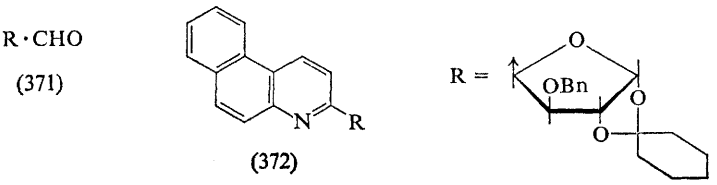


(370)

reaction of the appropriate carbonyl sugar derivatives with alkylidene triphenylphosphorane. The best results were obtained when the Wittig reagent was prepared from triphenylphosphonium methyl bromide and sodium amide in liquid ammonia.[514]

Suitable 3-keto-compounds may be useful starting materials for nucleophilic displacement reactions at C-2 in pyranoid derivatives.[271] Branched-chain sugars have been formed from ulose compounds and nitromethane.[277]

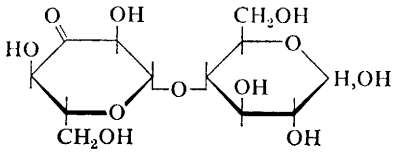
A Doebner-type of reaction between the *aldehydo*-sugar (371), β-naphthylamine, and acetoacetic acid afforded (372), and several related products.[515]



(371) (372)

Compound (373) was formed when cellobiose was incubated with a suspension of *Agr. tumefaciens*. Following enzymatic cleavage of unchanged cellobiose the keto-derivative was isolated by charcoal column chromatography.[516]

[513] P. M. Collins and P. Gupta, *Chem. Comm.*, 1969, 1288.
[514] D. G. Lance and W. A. Szarek, *Carbohydrate Res.*, 1969, **10**, 306.
[515] Yu. A. Zhdanov, Yu. E. Alekseev, and G. N. Dorofeenko, *Zhur. obshchei. Khim.*, 1969, **39**, 1413.
[516] K. Hayano and S. Fukui, *J. Biochem.* (*Japan*), 1969, **64**, 901.

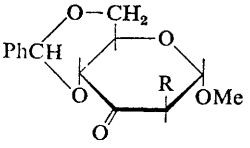


(373)

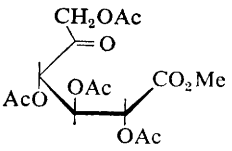
It has been shown that many mono- and di-saccharides form dicarbonyl compounds when treated with arsenite.[517] The role of 3-deoxy-glucosone as an intermediate in the browning of saké has been studied.[518]

The bis(2,4-dinitrophenylhydrazone) of 1,4:3,6-dianhydro-D-*threo*-hexitol-2,5-diulose gave an abnormally high specific rotation. Reasons for this were discussed.[519]

Compound (374) and its deuteriated derivative (375) have been prepared and examined by n.m.r. spectroscopy.[520] The results showed that the axial proton at C-2 resonates at lower field than the equatorial C-2 proton.



(374) R = H
(375) R = D



(376)

Compounds such as (376) have been prepared by oxidation of acetylated methyl glycosides with chromium trioxide in acetic acid[520a] (see also Chapter 22).

[517] E. Jellum, *Biochim. Biophys. Acta*, 1969, **170**, 430.
[518] S. Oka, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 554.
[519] P. M. Collins, P. T. Doganges, A. Kolarikol, and W. G. Overend, *Carbohydrate Res.*, 1969, **11**, 199.
[520] R. F. Butterworth, P. M. Collins, and W. G. Overend, *Chem. Comm.*, 1969, 378.
[520a] S. J. Angyal and K. James, *Chem. Comm.*, 1969, 617.

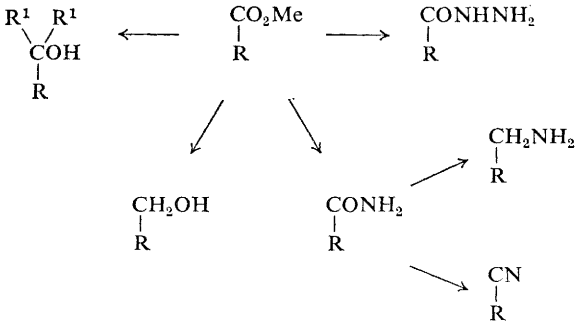
17
Sugar Acids and Lactones

Aldonic Acids

These will be treated generally in the order of increasing length of the carbon chains.

The n.m.r. and mass spectra of the methyl ester penta-acetate of 2-*O*-α-D-mannopyranosyl-D-glyceric acid, obtained as its sodium salt from a red alga, were used to confirm the assigned structure.[521] Complexes formed between aldotetronolactones and molybdic acid were examined in aqueous solution. O.r.d. curves showed a maximum at 330 nm, and the sign of the Cotton effects could be correlated with the C-2 configurations.[522] Aldonic acids have also been examined.[523] The absolute configurations of optically active 2,3-dihydroxybutyric acids (4-deoxy-threonic and -erythronic acids) have been examined by o.r.d. methods.[524]

A series of di-*O*-benzylidene-pentonic acid derivatives have been prepared (using standard reactions) as shown in Scheme 80.[525]



(R = di-*O*-benzylidene-tetrahydroxybutyl R^1 = Me, Et, or Ph)

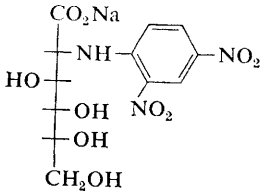
Scheme 80

[521] J. N. C. Whyte, *Canad. J. Chem.*, 1969, **47**, 4083.
[522] K. Takiura, M. Yamamoto, and S. Ohkawa, *J. Pharm. Soc. Japan*, 1969, **89**, 426.
[523] W. Voelter, E. Bayer, G. Barth, E. Bunnenberg, and C. Djerassi, *Chem. Ber.*, 1969, **102**, 2003.
[524] F. W. Bachelor and G. A. Miana, *Canad. J. Chem.*, 1969, **47**, 4089.
[525] H. Zurner, H. Voigt, and J. Voigt, *Carbohydrate Res.*, 1969, **9**, 5.

Acetals formed on condensation of D-ribono-1,4-lactone with cyclohexanone have been described,[212] and the commercially available 2,3-*O*-isopropylidene-D-gulono-1,4-lactone has been used in a synthesis of L-erythrose.[17] D-Glucono-1,5-lactone has been shown to adopt a non-chair conformation in the crystal;[526] complexes formed in solution between the free acid and titanium perchlorate have been studied.[527] Treatment of penta-*O*-acetyl-D-gluconyl chloride with silver perchlorate gave a gluconyl perchlorate which exploded on heating.[528] 2,3-Unsaturated hexono-1,5-lactone derivatives were obtained, somewhat surprisingly, on treatment of 3-deoxy-3-nitro-glycoside derivatives with alumina in refluxing toluene.[462]

Condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with mercuric cyanide in nitromethane gave the β-cyanide which was hydrolysed to 2,5-anhydro-D-allonic acid. This was then utilised in a synthesis of 8-β-D-ribofuranosyl adenine.[529]

Complexes formed between 2-amino-2-deoxy-D-gluconic acid and europium(III) have been examined by polarographic and n.m.r. methods,[530] and the sodium salt (377) of the *N*-(2,4-dinitrophenyl) derivative, on



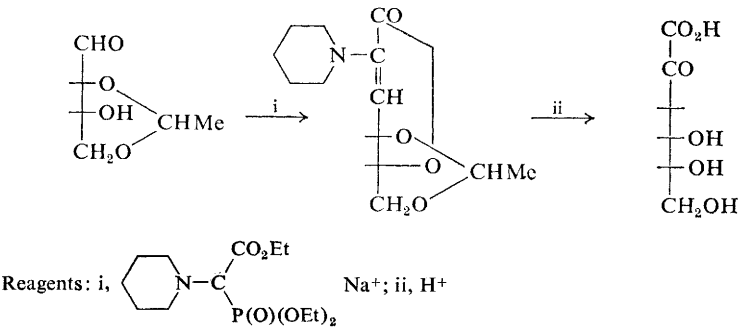
(377)

irradiation with u.v. light, gave D-arabinose. It was suggested that this procedure may be a useful complement to the ninhydrin degradation used for the identification of 2-amino-2-deoxy-aldoses.[18]

The configuration of strontium D-*gluco*-isosaccharinate has been determined crystallographically.[495] The separation of the sixteen heptono-1,4-lactones by g.l.c. is reported on p. 186.

A review has been published on the chemical and biochemical syntheses of 3-deoxy-glyculosonic acids,[530a] and several new reports on the preparation of members of this series have appeared, for example the D-*erythro*-hexose compound was obtained as illustrated in Scheme 81.[531] A related

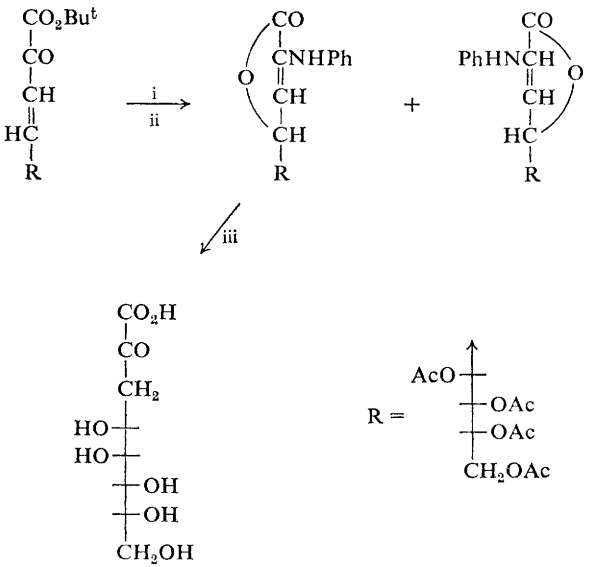
[526] M. L. Hackert and R. A. Jacobson, *Chem. Comm.*, 1969, 1179.
[527] C. G. Macarovici and L. Czeglédi, *Rev. Roumaine Chim.*, 1969, **14**, 57.
[528] Yu. A. Zhdanov, G. A. Korol'chenko, G. N. Dorofeenko, and G. G. Gatko, *Zhur. obshchei. Khim.*, 1969, **39**, 1128.
[529] M. Bobek and J. Farkaš, *Coll. Czech. Chem. Comm.*, 1969, **34**, 247.
[529a] H. Yamasaki and T. Hashizume, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1362.
[530] Y. Masuda and S. Misumi, *J. Chem. Soc. Japan*, 1969, **90**, 471.
[530a] B. A. Dmitriev and L. V. Bakinovskii, *Uspekhi Biol. Khim.*, 1968, **9**, 182 (*Chem. Abs.*, 1969, **70**, 78,261*a*).
[531] G. Baschang and H. Fritz, *Helv. Chim. Acta*, 1969, **52**, 300.



Reagents: i, $N{-}C(CO_2Et)P(O)(OEt)_2$ Na^+; ii, H^+

Scheme 81

Wittig procedure has been used to obtain the D-*manno*-octose analogue; the final stages of this synthesis are shown in Scheme 82.[532] A specific



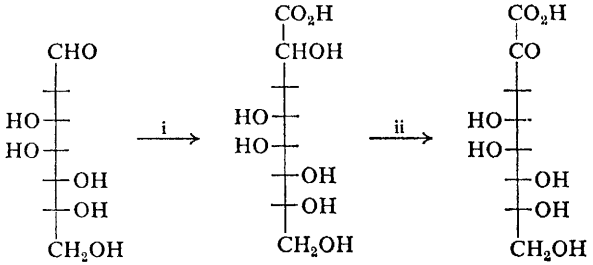
Reagents: i, CF_3CO_2H; ii, $PhNH_2 \cdot HCl$, PhN=CHPh; iii, MeOH, H^+

Scheme 82

oxidation, effected with vanadium chloride–potassium chlorate, has been applied to several 3-deoxyaldonic acids to obtain this and further 3-deoxyulosonic acids. The principle is outlined in Scheme 83,[533] and it has also

[532] N. K. Kochetkov, B. A. Dmitriev, and L. V. Backinowsky, *Carbohydrate Res.*, 1969, **11**, 193.

[533] D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 691.



Reagents: i, NaCN, H_2O; ii, vanadium catalysts

Scheme 83

been applied to obtain the D-*gluco*-[534] and the D-*galacto*-[535] isomers. These acids are found in hydrolysates of lipopolysaccharides, and a method, based on g.l.c. of TMS derivatives, has been developed for their analysis.[533]

2,3-Unsaturated cyclic compounds which are derivatives of ulosonic acids have been referred to in Chapter 14.

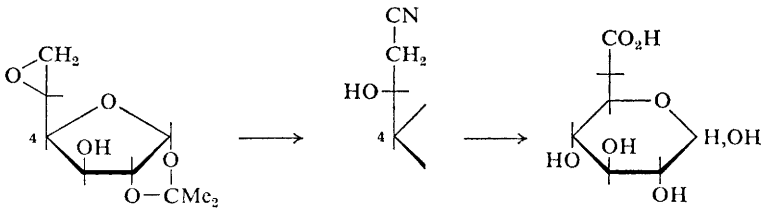
It has been shown that all 3-deoxy-ulosonic acids give a positive reaction with periodate–thiobarbituric acid, and that the test is not specific for neuraminic acid. This paper also reported the isolation of 3-deoxy-ulosonic acids from soya beans and from bananas, and recorded the synthesis of 3-deoxy-D-*glycero*-β-D-*galacto*-nonulosonic acid by standard procedures.[536]

Uronic Acids

The polyoxins, peptide nucleoside derivatives based on 5-amino-5-deoxy-D-alluronic acid, are mentioned on p. 67.

The use of L-glucuronic acid as an intermediate in the conversion of D-glucose to its enantiomer has been referred to in Chapter 2.[16]

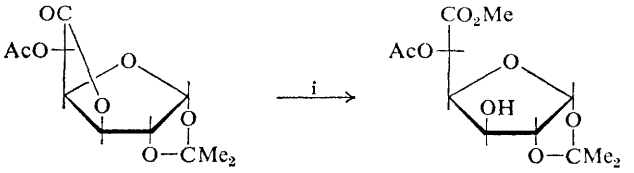
6-Deoxy-D-*gluco*-hepturonic acid was prepared as illustrated in Scheme 84, and was converted by use of standard reagents into a series of derivatives.[537] Other workers have developed a method for converting uronic



Scheme 84

[534] D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 983.
[535] M. B. Perry and A. C. Webb, *Canad. J. Biochem.*, 1969, **47**, 2893.
[536] W. Gielen, *Z. Naturforsch.*, 1968, **23b**, 1598.
[537] W. Meyer zu Reckendorf, *Chem. Ber.*, 1969, **102**, 2977.

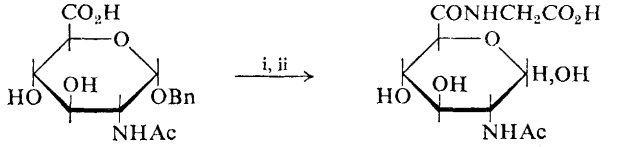
acid lactones into methyl esters, without affecting acetate esters or isopropylidene acetals (Scheme 85).[538] Amino-acid amides of aminouronic



Reagents: i, MeOH, Et_3N, AcOH

Scheme 85

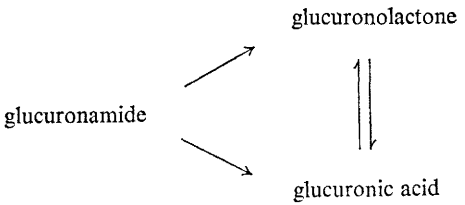
acid derivatives have been prepared as illustrated for the glycine derivatives, Scheme 86,[539] and the hydrolysis of the unsubstituted amide of D-glu-



Reagents: i, $H_2NCH_2CO_2Bn$, DCC; ii, H_2–Pd

Scheme 86

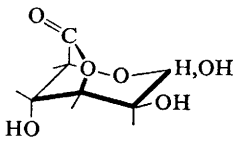
curonic acid was examined in buffered solutions; rate constants were determined for the reactions shown in Scheme 87.[540]



Scheme 87

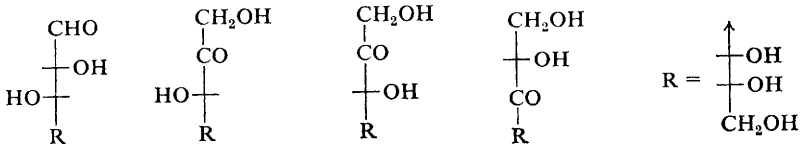
Detailed studies have been made of the polarographic reductions of D-glucurono-3,6-lactone at varying pH values.[541] The D-*manno*-isomer was also examined and was found to be reduced by a diffusion-controlled process, whereas a kinetic process was involved with the *gluco*-compound.[542] This was interpreted as indicating that the mannuronolactone ring (378) is the more stable, as would have been expected on conformational grounds.

[538] I. Matsunaga and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1383.
[539] J. Yoshimura, T. Sato, and H. Ando, *Bull. Chem. Soc. Japan*, 1969, **42**, 2352.
[540] T. Yamana, Y. Mizukami, and F. Ichimura, *J. Pharm. Soc. Japan*, 1969, **89**, 173.
[541] R. J. Thibert and J. R. Johnston, *Canad. J. Chem.*, 1969, **47**, 265.
[542] J. R. Johnston and R. J. Thibert, *Canad. J. Chem.*, 1969, **47**, 1433.



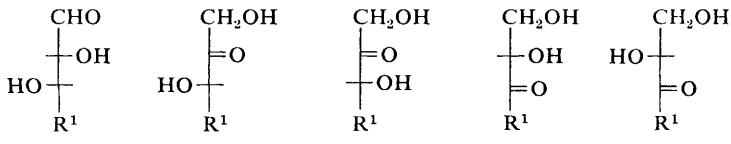
(378)

Continued interest has been shown in the isomerisation of uronic acids. The products formed on heating D-glucuronic acid (379) in aqueous solutions at pH 7 were separated by ion-exchange chromatography and identified as: D-*lyxo*-hex-5-ulosonic acid (380, main product), which is simply formed by isomerisation at C-1 and C-2, L-*ribo*-hex-5-ulosonic acid

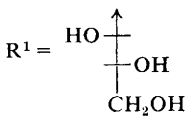


(379) (380) (381) (382)

(381), D-mannuronic, D-alturonic, and D-alluronic acid. L-Iduronic acid was not detected.[543] A further product was later identified as L-*ribo*-hex-4-ulosonic acid (382).[544] Similar treatment of D-galacturonic acid (383) gave mainly the corresponding keto compound (384) but the ulosonic acids (385)—(387) were also detected amongst the products together with D-taluronic, D-guluronic, and D-iduronic acids.[545]



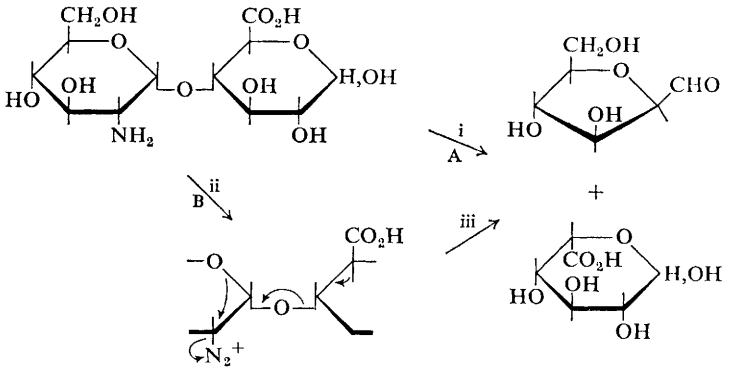
(383) (384) (385) (386) (387)



An interesting related isomerisation has been noted to occur during the deamination of heparin with nitrous acid. As well as cleaving the 2-amino-2-deoxy-D-glucopyranosyl bonds, the reagent caused epimerisation at C-5 of the glucuronic acid. A model experiment proceeded as shown in

[543] B. Carlsson, O. Samuelson, T. Popoff, and O. Theander, *Acta. Chem. Scand.*, 1969, **23**, 261.
[544] B. Carlsson and O. Samuelson, *Acta. Chem. Scand.*, 1969, **23**, 318.
[545] B. Carlsson and O. Samuelson, *Carbohydrate Res.*, 1969, **11**, 347.

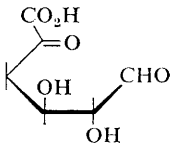
Scheme 88A.[546] No mechanism was proposed; we believe the reaction may have proceeded by way of a β-elimination as shown (Scheme 88B).



Reagents: i, HNO_2, AcOH; ii, HNO_2; iii, AcOH

Scheme 88

4,5-Unsaturated acids of the type proposed here as intermediates have been referred to in Chapter 14, and it has been reported that an enzyme from *A. niger* which hydrolyses the glycosidic linkages of 4,5-unsaturated galactopyranosides affords 4-deoxy-L-*threo*-hexos-5-ulose uronic acid (388) as would have been expected.[547]



(388)

2′,3′-*O*-Isopropylidene-nucleosides have been converted by alkaline permanganate into the 5′-carboxylic acid analogues for comparisons with purine nucleoside 5′-carboxylic acids obtained from polynucleotides,[548] and a 2′-deoxy-D-*erythro*-pentofuranosyl nucleoside on treatment with oxygen in the presence of a platinum catalyst also afforded the 5′-acid, which was much less sensitive to acidic hydrolysis than was the parent substance.[549]

D-Glucuronic acid formed a complex with molybdic acid in acetic acid which gave an o.r.d. maximum at 345 nm. When molybdic acid was added to the lactone a slow rotational change occurred, the production of the

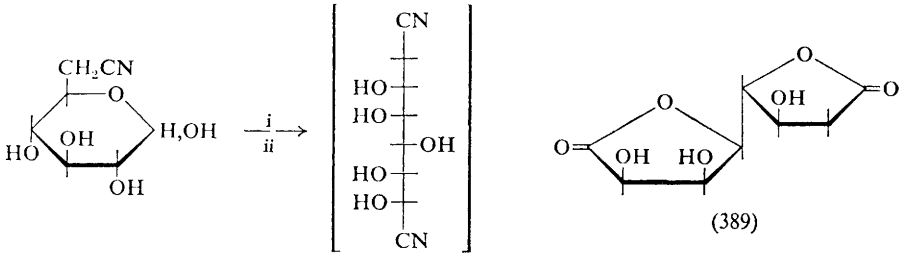
[546] F. Yamauchi, M. Kosakai, and Z. Yosizawa, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 721.
[547] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. Japan*, 1969, **43**, 139.
[548] P. E. Harmon, C. V. Zenarosa, and S. K. Gupta, *Chem. and Ind.*, 1969, 1141.
[549] K. C. Tsou, N. J. Santora, and E. E. Miller, *J. Medicin. Chem.*, 1969, **12**, 173.

free acid being recognisable by observation of the optical rotation at 345 nm.[550] C.d. features of uronic acids are noted on p. 182.

The mass spectra of isopropylidene acetals of several uronic acids and their derivatives have been measured, and the fragmentation patterns proposed;[551] free uronic acids and their lactones have also been examined (p. 179).

Aldaric Acids

The synthesis of the di-lactone (389) of 2-deoxy-L-*glycero*-L-*gulo*-octaric acid is outlined in Scheme 89.[552]



(389)

Reagents: i, NaCN; ii, H^+

Scheme 89

Efforts to produce inosose derivatives by the acyloin condensation of the tetra-*O*-trimethylsilyl derivatives of dimethyl galactarate were not successful although the required cyclisation was shown to have occurred by the isolation of the trimethylsilyl ether of catechol.[553]

Complexes formed between D-glucosaccharic acid and gallium and indium in aqueous solution have been found to be more stable than those formed from D-gluconic acid.[554]

Ascorbic Acid

Phosphorylation of 5,6-*O*-isopropylidene-L-ascorbic acid with phosphorus oxychloride in acetone gave the 2-phosphate, the 3-phosphate, the 3-pyrophosphate, and bis(L-ascorbic acid-3,3′-yl)phosphate.[555]

The kinetics of the oxidation of ascorbic acid by copper have been studied with reference to the effect on the reaction of added ions such as cyanide which complex with one of the oxidation states of copper preferentially. 1,10-Phenanthroline also slowed down the oxidation process.[556]

[550] K. Takiura, M. Yamamoto, and N. Tsujimoto, *J. Pharm. Soc. Japan*, 1969, **89**, 730.
[551] V. Kováčik, Š. Bauer, and P. Šipoš, *Coll. Czech. Chem. Comm.*, 1969, **34**, 2409.
[552] I. Dijong, *Carbohydrate Res.*, 1969, **11**, 428.
[553] D. Detert and B. Lindberg, *Acta. Chem. Scand.*, 1969, **23**, 690.
[554] C. G. Macarovici and E. Perte, *Rev. Roumaine Chim.*, 1969, **14**, 1113.
[555] H. Nomura, T. Ishiguro, and S. Morimoto, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 381, 387.
[556] A. Hanaki, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1839, 1964.

The kinetics of reduction of hexacyanoferrate ion to hexacyanoferrous by ascorbic acid have also been examined.[557]

A method for the spectrophotometric determination of ascorbic acid has been described, which permitted the analysis of 2×10^{-5} M of the compound.[558] An analytical study has been undertaken of the degradation products formed when ascorbic acid was heated in aqueous solution. Ten furan derivatives were detected as well as two lactones and 3-hydroxy-2-pyrone.[559]

A synthesis of L-*xylo*-hex-3-ulose from ascorbic acid has already been described.[15]

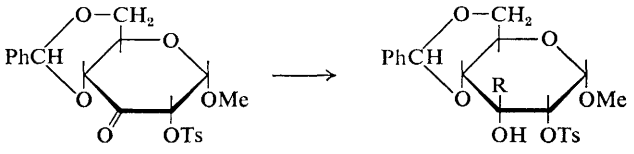
The separation of L-ascorbic acid by g.l.c. from its D-*arabino*-isomer is referred to on p. 186, and the crystal structure of sodium ascorbate is noted on p. 180.

[557] U. S. Mehrotra, M. C. Agrawal, and S. P. Mushran, *J. Phys. Chem.*, 1969, **73**, 1996.
[558] Y. Ogata and Y. Kosugi, *Bull. Chem. Soc. Japan*, 1969, **42**, 2282.
[559] J. H. Tatum, P. E. Shaw, and R. E. Berry, *J. Agric. Food Chem.*, 1969, **17**, 38.

18
Inorganic Derivatives

Carbon-bonded Compounds

For the first time cyclic sugar analogues have been prepared having phosphorus as the ring hetero-atom.[429] Other carbon–phosphorus bonded compounds have been prepared by reaction of suitably protected ulose derivatives with dimethyl phosphite and sodium methoxide in benzene as illustrated in Scheme 90. Structural assignments were carried out by n.m.r. studies of ^{31}P–^{1}H couplings.[560]



R = $PO(OMe)_2$

Scheme 90

Reaction of acylglycosyl halides with magnesium in ether has been reported to yield the appropriate Grignard reagents. Reactions of these products were not described.[560a] Grignard reagents prepared from chloromethyltrichlorosilane reacted with tetra-acetylglucosyl chloride to give, after hydrolysis, polymeric carbohydrate products having CH_2SiO groups at the anomeric positions.[561]

Oxygen-bonded Compounds

The use of complexes, formed by oxygen bonding between carbohydrates and reagents used for reacting with carbonyl compounds, to obtain asymmetric products has been referred to.[192, 193]

A crystalline 1 : 1 adduct of methyl α-D-glucopyranoside and potassium hydrogen carbonate has been prepared by treating an aqueous potassium

[560] L. Evelyn, L. D. Hall, P. R. Steiner, and D. H. Stokes, *Chem. Comm.*, 1969, 576.
[560a] L. Maijs and G. Fisers, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1968, 756 (*Chem. Abs.*, 1969, **70**, 68,658*w*).
[561] L. Maijs and G. Fisers, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1968, 756 (*Chem. Abs.*, 1969, **70**, 78,321*v*).

hydroxide solution of the glycoside with carbon dioxide, or by use of equimolar proportions of the glycoside and potassium hydrogen carbonate in water. N.m.r. parameters of the product were compared with those of the free compound.[562]

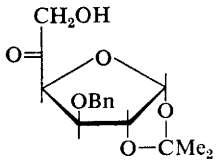
A series of complexes formed between aldonic acids and various inorganic complexing agents have already been noted as follows: 2-amino-2-deoxy-D-gluconic acid with europium(III),[530] glucuronic acid[550] and aldotetronolactones[522] with molybdic acid, glucosaccharic acid with gallium and indium,[554] and gluconic acid with titanium perchlorate.[527]

[562] J. W. Berry, B. I. Mayall, J. T. Marrel, and A. J. Deutschman jun., *Carbohydrate Res.*, 1969, **9**, 122.

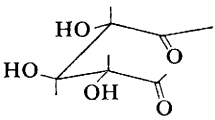
19
Cyclitols

The IUPAC–IUB tentative rules on the nomenclature of cyclitols mentioned in volume 2 (p. 162) have now appeared in other journals.[563, 564] A system for numbering asymmetric substituted *myo*-inositols has been proposed.[565]

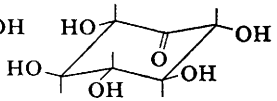
It is known that *myo*-inositol arises in nature directly from D-glucose without rearrangement of its carbon skeleton, and it has been suggested that D-*xylo*-hexos-5-ulose 6-phosphate may be a reaction intermediate that can undergo an intramolecular aldol condensation to give a 6-membered carbocyclic product. D-*xylo*-Hexos-5-ulose (391) which had been prepared by sequential catalytic hydrogenolysis and hydrolysis of (390)



(390)



(391)



(392)

afforded, on treatment with dilute alkali, *myo*-inosose (392), which was characterised as its reduction products [566] (see vol. 2, p. 163). Compound (392) underwent epimerisation to give the products with axial hydroxy-groups on either side of the carbonyl group. This work adds considerable evidence for the postulated biosynthetic path, and represents the second chemical synthesis of *myo*-inositol from D-glucose (the other being achieved *via* 6-deoxy-6-nitro-D-glucose).

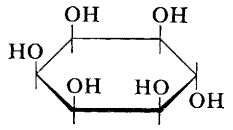
A new method for the synthesis of 1,2-*O*-isopropylidene-*myo*-inositol has been described using 2,2-dimethoxypropane in hot DMSO in the presence of toluene-*p*-sulphonic acid.[567] The product was converted into 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol by sequential benzylation, acidic hydrolysis, selective allylation of the free equatorial hydroxy-group,

[563] *Biochem. J.*, 1969, **112**, 17.
[564] *Z. physiol. Chem.*, 1969, **350**, 523.
[565] B. A. Klyashchitskii, V. I. Shvets, and N. A. Preobrazhenskii, *Zhur. org. Khim.*, 1969, **5**, 192.
[566] D. E. Kiely and H. G. Fletcher jun., *J. Org. Chem.*, 1969, **34**, 1386.
[567] R. Gigg and C. D. Warren, *J. Chem. Soc.* (*C*), 1969, 2367.

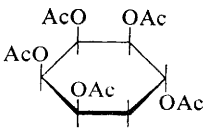
benzylation, and removal of the allyl group. The 1-phosphate, a possible intermediate in the synthesis of phosphatidyl-inositol, was amongst several derivatives of pentabenzyl-*myo*-inositol that were prepared. 3,4,5,6-Tetra-*O*-benzyl-*myo*-inositol [568] and 1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol [569] have also been described.

The structure, synthesis, and biochemistry of inositol phosphatides have been reviewed.[570] Methylation of phytic acid (*myo*-inositol hexaphosphate) with diazomethane in methanol afforded the crystalline dodecamethyl ester.[571] The separation of partially phosphorylated inositols and their methyl esters was discussed. A brief survey was given of the occurrence in nature of partially phosphorylated *myo*-inositol esters.[572] Methods were assessed for the phosphorylation of monosubstituted inositols, and the only reagent found to be satisfactory was the little-used *N*-benzoylphosphoramidic acid, $PhCONHPO_3H_2$, employed as its triethylammonium salt in DMF. With this reagent *myo*-inositol 1,2,4,5,6- and 1,2,3,4,5-pentaphosphates were synthesised from 1-benzyl-*myo*-inositol and 4-benzyl-*myo*-inositol respectively. After phosphorylation the benzyl ethers were cleaved by hydrogenolysis.

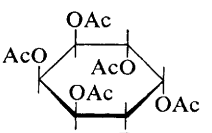
The reaction of DL-*epi*-inositol (393) with acetyl bromide and acetic anhydride gave four bromo-compounds, two of which, (394) and (395),



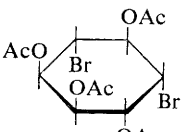
(393)



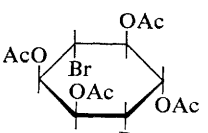
(394)



(395)



(396)

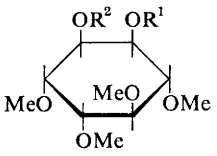


(397)

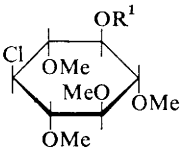
were known previously. The structures of the other two, (396) and (397), were established by n.m.r. studies, and by conversion into deoxy compounds.[573]

[568] T. P. Zubkova, Z. Ya. Khrapkova, I. K. Sarycheva, and N. A. Preobrazhenskii, *Zhur. org. Khim.*, 1968, **4**, 2226.
[569] B. A. Klyashchitskii, V. V. Pimenova, V. I. Shvets, and N. A. Preobrazhenskii, *Zhur. obshchei. Khim.*, 1969, **39**, 1653.
[570] B. A. Klyashchitskii, S. D. Sokolov, and V. I. Shvets, *Uspekhi Khim.*, 1969, **38**, 740 (*Chem. Abs.*, 1969, **71**, 22,268*m*).
[571] S. J. Angyal and A. F. Russell, *Austral. J. Chem.*, 1969, **22**, 383.
[572] S. J. Angyal and A. F. Russell, *Austral. J. Chem.*, 1969, **22**, 391.
[573] T. Suami, A. Suzuki, M. Uchida, and S. Yanagida, *Bull. Chem. Soc. Japan*, 1969, **42**, 2672.

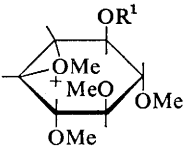
The reaction of (398) or (399) with phosphorus pentachloride gave (401) and (402). It was suggested that the ions (403) and (404) were intermediates,



(398) R^1 = Me, R^2 = H
(399) R^1 = Bz, R^2 = H
(400) R^1 = Bz, R^2 = Me



(401) R^1 = Me
(402) R^1 = Bz

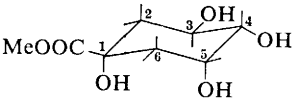


(403) R^1 = Me
(404) R^1 = Bz

thus providing another example of participation by an 'inert' group such as methoxy. It was also observed that the benzoyl group in the monobenzoate (399), obtained by selective benzoylation of the diol, underwent migration on methylation with methyl iodide and silver oxide to yield (400).[574]

The reduction of DL-*epi*-inosose-2 with sodium borohydride has been studied at various pH values. At pH 8·5 *epi*-inositol was formed in high yield.[575]

The order of reactivity of the hydroxy-groups in methyl quinate (405) towards acylating agents has been investigated. Towards benzoyl chloride



(405)

the order of reactivity was 4>5>3, and towards toluene-*p*-sulphonyl chloride, 4 = 5>3. Since the normal pattern of equatorial hydroxy-groups being more reactive than axial hydroxy-groups was not followed, it was suggested that the 5-hydroxy-group was activated by hydrogen-bonding to the 1-hydroxy-group.[576]

Derivatives of the three diastereoisomeric 1,2,3-cyclopentanetriols and three diastereoisomeric 1,2,4-cyclopentanetriols have been synthesised *via* reaction sequences starting with cyclopenten-2-ol, cyclopenten-3-ol, and various cyclopentenediols.[577] The four 1,2-anhydro-cyclopentanetriols, their acetylated derivatives, and several substituted cyclopentane epoxides were also prepared. The kinetics of the acid-catalysed hydrolysis of the

[574] G. E. McCasland, M. O. Naumann, and L. J. Durham, *J. Org. Chem.*, 1969, **34**, 1382.
[575] R. Suemitsu and S. Komiyama, *Doshisha Daigaku Rikogaku Kenkyu Hokoku*, 1968, **9**, 177 (*Chem. Abs.*, 1969, **70**, 11,545*l*).
[576] D. Mercier, J. Cléophax, J. Hildesheim, A. M. Sépulchre, and S. D. Géro, *Tetrahedron Letters*, 1969, 2497.
[577] R. Steyn and H. Z. Sable, *Tetrahedron*, 1969, **25**, 3579.

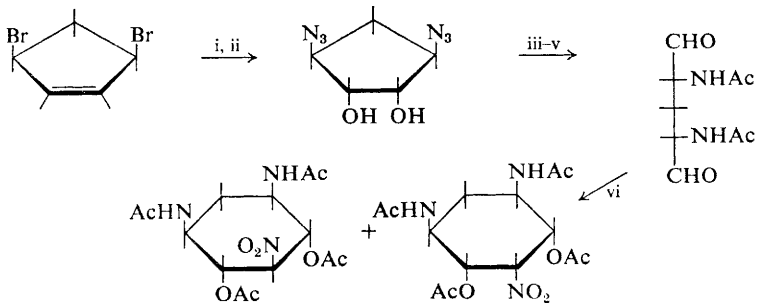
epoxides were studied. The presence or absence of intramolecular hydrogen bonds, determined by i.r. spectroscopy, was useful in configurational assignments of some of the compounds. The frequency of an i.r. absorption band near 840 cm^{-1}, which is characteristic of an oxiran ring, was correlated with the position and orientation of the substituent in a number of the monosubstituted cyclopentane-epoxides.

Sephadex gel chromatography of inositol phosphates has been reported.[578] Efforts to produce inosose derivatives by the acyloin condensation of the tetra-*O*-trimethylsilyl derivative of dimethyl galactarate were not successful.[553]

Nitrogen-containing Derivatives

Five 1-anilino-2,3,4-triacetoxy-cyclopentanes have been prepared and characterised.[579]

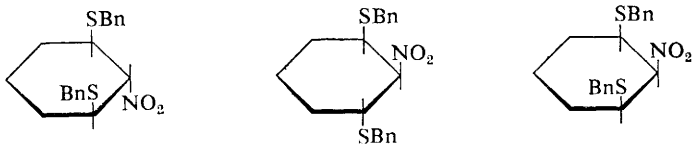
Diamino-nitro-cyclohexanediol derivatives have been synthesised[580] (Scheme 91). The results and implications of these, and of many other reactions, were discussed in detail.



Reagents: i, NaN_3; ii, $KMnO_4$; iii, H_2–Pd; iv, Ac_2O; v, IO_4^-; vi, CH_3NO_2, MeOH, MeONa

Scheme 91

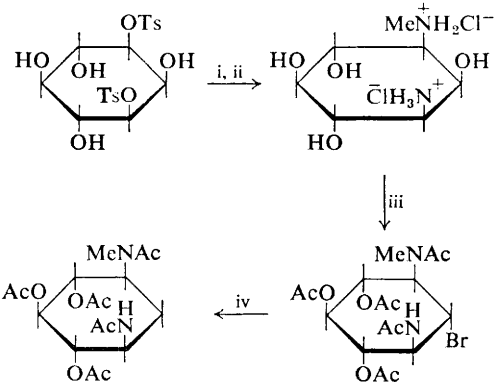
2-Nitro-cyclohexanedithiols with *trans* (406), *chiro* (407), and *cis* (408) configurations have been formed by nitromethane cyclisation of glutaraldehyde in the presence of α-toluenethiol.[581]



(406) (407) (408)

[578] J. H. Steward and M. E. Tate, *J. Chromatog.*, 1969, **45**, 400.
[579] G. Kreze and R. Rubner, *Chem. Ber.*, 1969, **102**, 1280.
[580] A. Hasegawa and H. Z. Sable, *Tetrahedron*, 1969, **25**, 3567.
[581] F. W. Lichtenthaler and P. Voss, *Annalen*, 1969, **724**, 81.

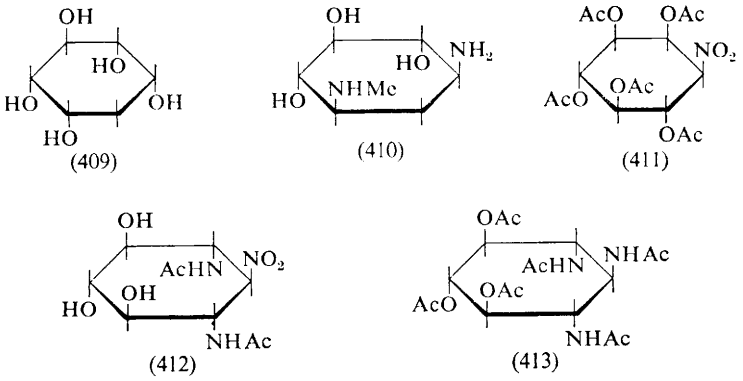
Two syntheses of hyosamine (*N*-methyl-2-deoxystreptamine), a component of the antibiotics hygromycin B and destomycin A, have been reported. DL-Hyosamine has been prepared as illustrated [581a] (Scheme 92).



Reagents: i, $MeNHNH_2$; ii, HCl; iii, AcBr, Ac_2O; iv, H_2

Scheme 92

(−)-Hyosamine has been prepared by a route previously described for the racemic compound [582] (*Annalen*, 1965, **689**, 235) except that *Acetobacter suboxydans* was used for the initial oxidation step of (409). The absolute configuration of (−)-hyosamine was established as (410).

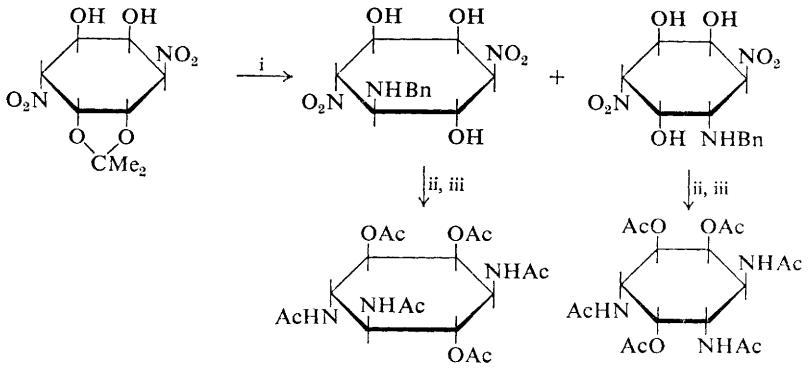


Triamino-trideoxy-inositols have been prepared from nitroinositols, *e.g.* (411), by reaction with ammonia followed by *N*-acetylation to yield nitro diacetamido derivatives, *e.g.* (412), and then sequential reduction and acetylation to yield vicinal triacetamido compounds, *e.g.* (413).[360]

[581a] T. Suami and H. Sano, *Tetrahedron Letters*, 1969, 1795.

[582] N. Kurihara, K. Hayashi, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 256.

The syntheses of an *as*-inosatriamine and other inosatriamine derivatives (Scheme 93) have been described, and structures assigned by n.m.r. methods.[583]



Reagents: i, $BnNH_2$; ii, Pd–C, MeOH, AcOH; iii, acetylation

Scheme 93

The deamination of inosamines with nitrous acid gave products from which only small amounts of inositols were isolated. However, nitrous acid treatment of inosamine penta-acetates gave good yields of inositols as their penta-acetates. Some reactions proceeded with complete inversion and others mainly with retention of configuration. Elimination also occurred to some degree, affording enolacetates.[584]

[583] F. W. Lichtenthaler and P. Voss, *Tetrahedron Letters*, 1969, 2297.
[584] S. J. Angyal and J. S. Murdoch, *Austral. J. Chem.*, 1969, **22**, 2417.

20
Antibiotics

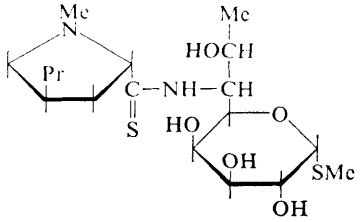
The proceedings of a symposium on gentamicin have been published.[585] Full details of the total syntheses of kanamycins A,[586, 586a] B,[587] and C[588] have been given (see vol. 2, pp. 171, 172). A series of *N*-alkyl derivatives of paromomycin and neomycin have been described, including the phenylethyl derivative which had marked activity.[589] A preliminary report has been published on what is claimed to be the first-known phosphoramido-amino sugar antibiotic (possibly a derivative of neomycin C).[590] The n.m.r. spectra of dihydrostreptomycin and of some of its derivatives have been studied, and the α-linkages in the molecule confirmed.[591] A biosynthetic precursor of streptomycin, 'compound L', has been shown to be a monophosphorylated derivative, with the phosphate group on C-6 of the streptidine moiety.[592]

Full details of the synthesis of methyl kasugaminide have been given [592a] (*cf.* vol. 2, p. 175); another route tried involved reduction of an oxime at C-2 (*cf.* p. 68).

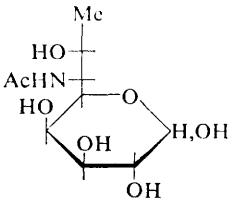
A series of C-7-esters of lincomycin has been prepared *via* the 2,3,4-tri-*O*-TMS derivatives. All had reduced antibiotic activity relative to the parent.[593] 7-Thio-lincomycins (both epimers) have been synthesised, one from a 6,7-epimine, the other by a direct displacement on a known 7-chloro-7-deoxy-derivative. Thioamide derivatives, such as (414), were also described.[594] Again the activity of these compounds was lower than that of the

[585] *J. Infect. Diseases*, 1969, **119**, Nos. 4/5.
[586] S. Umezawa, K. Tatsuta, and S. Koto, *Bull. Chem. Soc. Japan*, 1969, **42**, 533.
[586a] A. Hasegawa, N. Kurihara, D. Nishimura, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1968, **32**, 1123, 1130.
[587] S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura, and T. Tsumura, *Bull. Soc. Chim. Japan*, 1969, **42**, 537.
[588] S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, *Bull. Chem. Soc. Japan*, 1969, **42**, 529.
[589] L. Penasse, P. Barthelemy, and G. Nomine, *Bull. Soc. chim. France*, 1969, 2391.
[590] M. K. Majumdar and S. K. Majumdar, *J. Antibiotics*, 1969, **22**, 174.
[591] P. Claes, H. Vanderhaeghe, J. Totté, and G. Slinckx, *Bull. Soc. chim. belges*, 1969, **78**, 151.
[592] R. Nomi and O. Nimi, *Agric. Biol. Chem. (Japan)*, 1969, **33**, 1459.
[592a] K. Kitahara, S. Takahashi, H. Shibata, N. Kurihara, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 748.
[593] A. A. Sinkula, W. Morozowich, C. Lewis, and F. A. MacKellar, *J. Pharm. Sci.*, 1969, **58**, 1389.
[594] B. J. Magerlein and F. Kagan, *J. Medicin. Chem.*, 1969, **12**, 974.

parent. The reactivity of the hydroxy-groups in various partially substituted lincomycin derivatives towards acylation with valeric anhydride in pyridine has been studied,[595] and found to be 3-OH > 2-OH > 7-OH > 4-OH, but it would be dangerous to assume that this order represented the order in the unsubstituted antibiotic. Lincomycin has been found to be deactivated by *Streptomyces rochei*, probably by phosphorylation at the C-3-OH.[596]

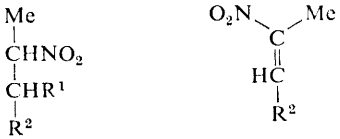


(414)

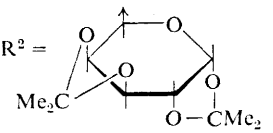


(415)

Jones and his group have carried out studies directed at the synthesis of *N*-acetyl-lincosamine (415), the *N*-acetyl derivative of the carbohydrate component of lincomycin. Addition of nitroethane to the 6-aldehyde of di-*O*-isopropylidene-D-galactopyranose gave a mixture of alcohols (416), which on acetylation gave the acetates (417). Treatment of these with



(420)

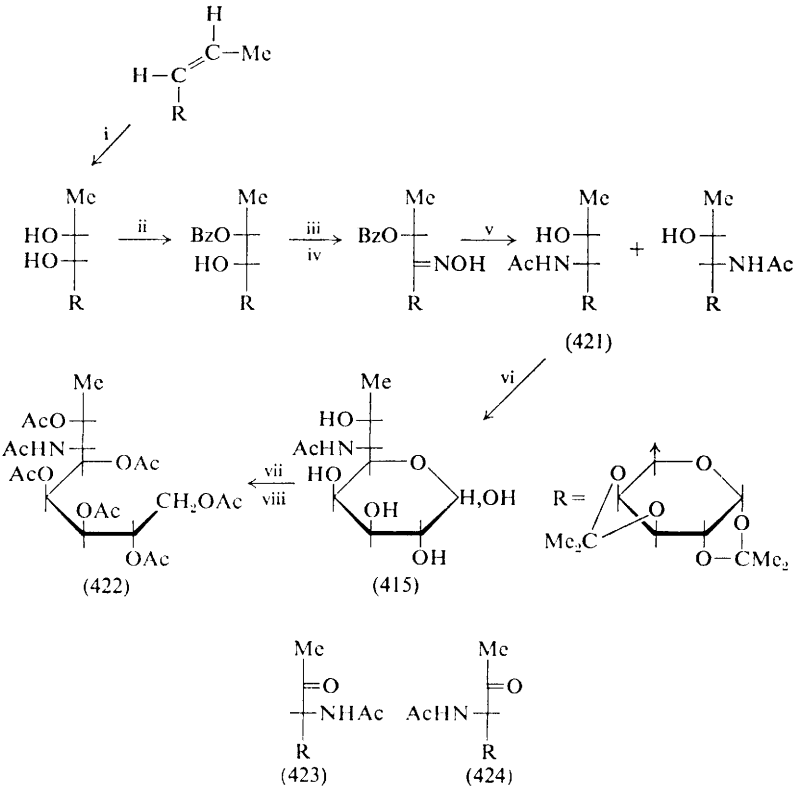


(416) $R^1 = OH$ (418) $R^1 = NH_2$
(417) $R^1 = OAc$ (419) $R^1 = NHAc$

triethylamine in benzene gave the *cis*-olefin (420) and its *trans* isomer in a ratio of 6 : 1. Addition of ammonia to (420) gave a mixture of nitroamines (418) that were *N*-acetylated to a 1 : 1 mixture of *N*-acetyl derivatives (419).[597]

A successful synthesis of *N*-acetyl-lincosamine (415) has been developed,[598] as shown in Scheme 94. It was reduced and acetylated to give (422) which had also been prepared from the natural product. Compound (421) was also prepared from (419) by oxidation with potassium permanganate to give the ketones (423) and (424). Reduction of (424) with sodium borohydride gave a mixture of epimeric alcohols, one of which was (421).

[595] W. E. Hamlin, *J. Pharm. Sci.*, 1969, **58**, 1291.
[596] A. D. Argoudelis and J. H. Coats, *J. Antibiotics*, 1969, **22**, 343.
[597] G. B. Howarth, D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 75.
[598] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *Chem. Comm.*, 1969, 1339.



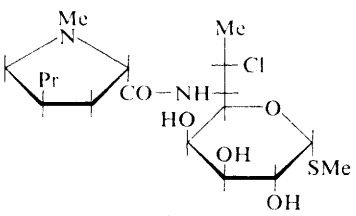
Reagents: i, aq $KMnO_4$; ii, PhCOCl(×1), pyr; iii, RuO_4; iv, $NH_2OH \cdot HCl$; v, LAH; vi, H^+; vii, $NaBH_4$; viii, Ac_2O, pyr

Scheme 94

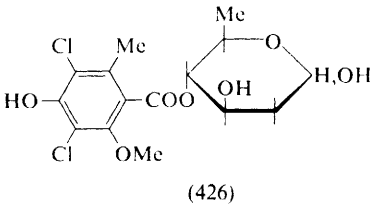
Extensions of fermentations, which usually afforded lincomycin, have yielded a sulphoxide and the free sugar derivative formed by removal of the methylthio-group. They were formed from lincomycin and had similar biological spectra to it.[599] Clindamycin, (425), produced by chlorination of lincomycin was *N*-demethylated by *Streptomyces punipalus*, whereas *S. armentosus* gave primarily the sulphoxide of (425).[600]

Everninocin I has been shown to be identical with curacin, a hydrolysis product of curamycin and of avilamycin, and has structure (426).[601] Everninose, a non-reducing disaccharide formed by hydrolysis of everninomicin D, has been shown to have structure (427). Prolonged hydrolysis gave the component monosaccharides which were identified; the anomeric

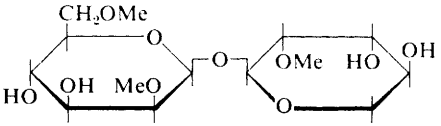
[599] A. D. Argoudelis and D. J. Mason, *J. Antibiotics*, 1969, **22**, 289.
[600] A. D. Argoudelis, J. H. Coats, D. J. Mason, and O. K. Sebek, *J. Antibiotics*, 1969, **22**, 309.
[601] H. Reimann, R. S. Jaret, and O. Z. Sarre, *J. Antibiotics*, 1969, **22**, 131.



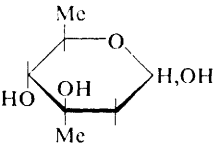
(425)



(426)



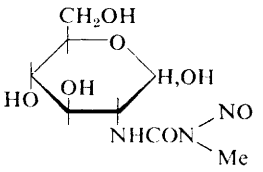
(427)



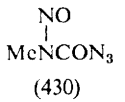
(428)

linkages were assigned on the basis of Klyne's Rule.[601a] Evermicose, a component of everninomicins B and D, has been shown to have structure (428), *i.e.* it is 'D-3-*epi*-mycarose'.[602]

Streptozotocin (429) has been synthesised by two routes, (i) reaction of 2-amino-2-deoxy-D-glucose with (430), and (ii) from tetra-*O*-acetyl-2-amino-2-deoxy-D-glucose by reaction with methyl isocyanate, then nitrosyl

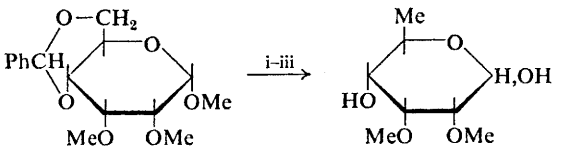


(429)



(430)

chloride, and finally deacetylation.[603] Further details have been given of the tuliposides[604] (see vol. 2, p. 49). A new synthesis of mycinose, a component of chalcomycin and of tylosin, has been achieved, as shown in Scheme 95.[605] L-Gulose and 3-*O*-carbamoyl-D-mannose have been found [11]



Reagents: i, NBS, CCl_4; ii, LAH; iii, N-H_2SO_4

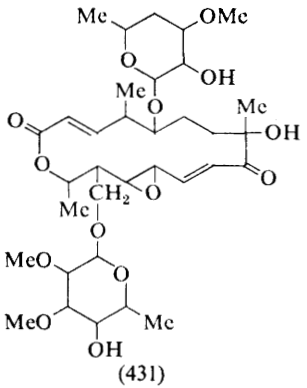
Scheme 95

[601a] A. K. Ganguly, O. Z. Sarre, and J. Morton, *Chem. Comm.*, 1969, 1488.
[602] A. K. Ganguly and O. Z. Sarre, *Chem. Comm.*, 1969, 1149.
[603] E. Hardegger, A. Meier, and A. Stoos, *Helv. Chim. Acta*, 1969, **52**, 2555.
[604] R. Tschesche, F.-J. Kammerer, and G. Wulff, *Chem. Ber.*, 1969, **102**, 2057.
[605] J. S. Brimacombe, O. A. Ching, and M. Stacey, *J. Chem. Soc.* (*C*), 1969, 197.

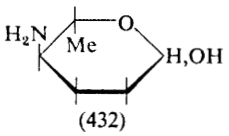
for the first time in natural products as components of the antibiotic bleomycin A_2.

Cirramycin A_1 is the first macrolide antibiotic to be studied that has mycaminose as the only sugar component. Many derivatives were prepared, some of which had improved biological properties.[606] The structures of olivomose, olivose, and oliose have been determined.[442] Structure (431) has been proposed for the mycinose- and chalcose-containing macrolide antibiotic, neutramycin.[607]

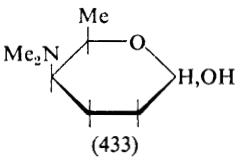
Tolyposamine, a component of tolypomycin, has been shown to have structure (432),[608] and ossamine, a component of ossamycin, has structure (433), which was confirmed by synthesis.[609] Daunomycin analogues have



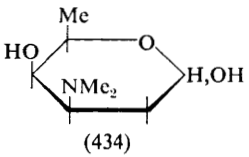
(431)



(432)



(433)

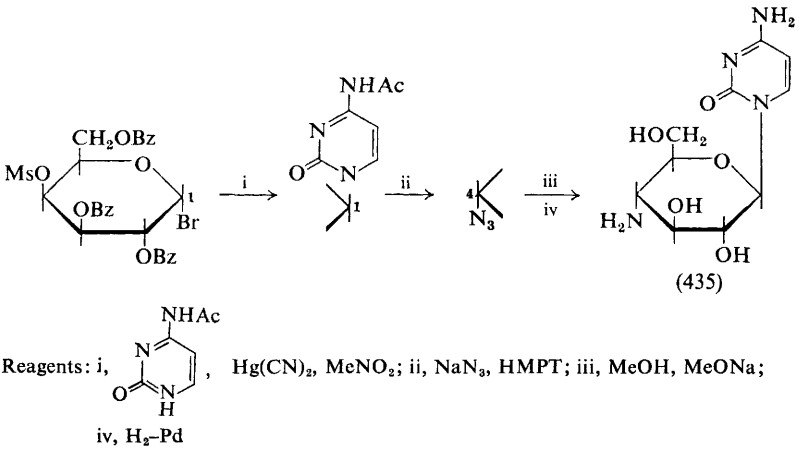


(434)

been synthesised in which the daunosaminyl moiety has been replaced by glucopyranosyl and 2-amino-2-deoxy-glucopyranosyl groups.[610] D-Rhodosamine, a sugar obtained from megalomicins A, B, C_1, and C_2, has been shown to be (434).[309] The complete structure of megalomicin A has been reported.[611]

[606] H. Tsukiura, M. Konishi, M. Saka, T. Naito, and H. Kawaguchi, *J. Antibiotics.*, 1969, **22**, 89, 100.
[607] L. A. Mitscher and M. P. Kunstmann, *Experientia*, 1969, **25**, 12.
[608] T. Kishi, S. Harada, M. Asai, M. Muroi, and K. Mizuno, *Tetrahedron Letters*, 1969, 97.
[609] C. L. Stevens, G. E. Gutowski, C. P. Bryant, R. P. Ginski, O. E. Edwards, and G. M. Sharma, *Tetrahedron Letters*, 1969, 1181.
[610] S. Penco, *Chim. Ind.* (*Milan*), 1968, **50**, 908 (*Chem. Abs.*, 1969, **70**, 11,953*j*).
[611] A. K. Mallams, *J. Amer. Chem. Soc.*, 1969, **91**, 7506.

A further report on the structure of showdomycin (*X*-ray method) has appeared [612] (*cf.* vol. 2, p. 163). 1-(4-Amino-4-deoxy-β-D-glucopyranosyl)-cytosine (435) has been synthesised (Scheme 96) and shown to be identical



Scheme 96

with a degradation product of gougerotin, thus establishing the structure of that antibiotic.[613]

Details of the syntheses of the pyrrole-[2,3-*d*]-pyrimidine nucleoside antibiotics, toyocamycin, sangivamycin, and tubercidin, have been described.[614] (*cf.* vol. 2, p. 179).

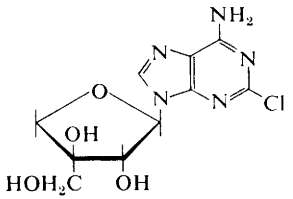
[612] Y. Tsukuda, T. Sato, M. Shiro, and H. Koyama, *J. Chem. Soc.* (*B*), 1969, 843.
[613] K. Watanabe, M. P. Kotick, and J. J. Fox, *Chem. and Pharm. Bull.* (*Japan*), 1969, **17**, 416.
[614] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1969, **91**, 2102.

21
Nucleosides

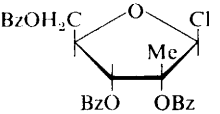
A very detailed paper has appeared which discussed at length the allowed and preferred conformations of nucleosides, nucleoside phosphates, nucleic acids, and polynucleotides,[615] and a Russian review of the structures and conformations of nucleosides, nucleotides, and their derivatives has also been published.[616] Reviews have appeared which have extended earlier lists of synthetic nucleosides [616a] and which have dealt particularly with methods for synthesising 1,2-*cis*-nucleosides.[617] 3′-Acetamido-3′-deoxy-adenosine has been isolated [617a] from culture filtrates of *Helminthosporium* sp. 215.

Synthesis

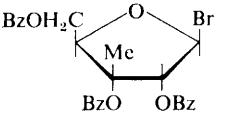
The synthesis of 9-(β-D-apio-L-furanosyl)-2-chloroadenine (436) has been reported,[618] as well as of other branched-chain nucleosides (see refs. 489, 496, 497, 501, and 502). A series of branched-chain cytidine and uridine



(436)



(437)



(438)

nucleosides which showed antiviral activity have been prepared using the glycosylating agents (437) and (438).[619]

The synthetic utility of trifluoroacetyl as an *N*-protective group has been demonstrated by the synthesis of purine nucleosides of 2-amino-2-deoxy-sugars.[619a] Purine nucleosides are unstable to acid, and thus it was

[615] M. Sundaralingam, *Biopolymers*, 1969, **7**, 821.
[616] N. N. Preobrazhenskaya and Z. A. Shabarova, *Uspekhi Khim.*, 1969, **38**, 222.
[616a] H. G. Garg, *J. Sci. Ind. Res. India*, 1969, **28**, 112.
[617] H. G. Fletcher jun., *Trans. N.Y. Acad. Sci.*, 1968, **30**, 649.
[617a] R. J. Suhadolnik, B. M. Chassy, and G. R. Waller, *Biochim. Biophys. Acta*, 1969, **179**, 258.
[618] F. Perini, F. A. Carey, and L. Long jun., *Carbohydrate Res.*, 1969, **11**, 159.
[619] E. Walton, S. R. Jenkins, R. F. Nutt, and F. W. Holly, *J. Medicin. Chem.*, 1969, **12**, 306.
[619a] M. L. Wolfrom and P. J. Carigliaro, *Carbohydrate Res.*, 1969, **11**, 63.

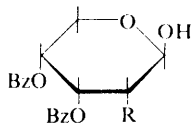
convenient to use methanolic ammonia to remove both *O*-acetyl and *N*-trifluoroacetyl groups in an otherwise conventional nucleoside synthesis. Attention was drawn to the fact that the *N*-trifluoroacetyl group participated at C-1 to a much lesser extent than did *N*-acetyl, and consequently both α- and β-nucleosides were obtained.

The syntheses of the following nucleosides have been reported: the anomeric forms of 9-(2-amino-2-deoxy-D-xylofuranosyl)adenine,[620] 6-substituted-9-(β-L-fucopyranosyl)purines,[621] a series of 4-substituted-6-pyrimidone ribofuranosyl nucleosides,[622] nicotinimide ribosides,[623] 7α, 7β-, and 1β-D-ribofuranosylhypoxanthine,[624] several diamino- and triamino-nucleosides,[625] 5-ethyl-2′-deoxyuridine containing a tritium label at C-4,[626] L-uridine,[627] derivatives of 2-chloroadenosine,[627a] 5′-carboxymethyl derivatives of adenosine, guanosine, cytidine, and uridine,[173] a uronic-acid-containing nucleoside,[549] an unsaturated nucleoside,[476] various *S*-nucleosides,[628] a triazine nucleoside,[629] and 8-β-D-ribofuranosyl-adenine.[529] Two bis(β-D-glucopyranosyl)hypoxanthines (with 1,7 and 1,9 links), and two β-D-glucopyranosyl-hypoxanthines (7- and 9-) have been described.[529a]

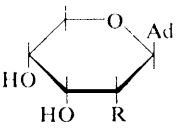
Thymine, cytosine, and uracil nucleosides have been prepared [291] from the crystalline halogeno-sugar (88). Nucleosides have been prepared from 2-deoxy-2-fluoro-pentoses.[303]

A method for the direct synthesis of pyrimidine nucleosides has been described in which the bases and glycosyl halides were heated in a solvent in the presence of mercuric cyanide. Variations in solvent gave rise to different products, such as bis-glycosides.[630, 631] The mercuric cyanide method was considered to be an important improvement on the standard synthesis of pyrimidine nucleosides and yields of up to 80% have been reported.[632] A method for synthesising nucleosides from sugars unprotected at the anomeric position has been investigated.[633] The reaction between adenine and (439) in the presence of polyphosphoric acid phenyl ester, followed by debenzoylation, afforded (441). Similarly, (442) and its α-anomer were obtained from adenine and (440), but in both cases yields were only moderate. Treatment of (439), with polyphosphoric acid phenyl

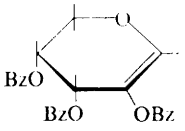
[620] M. L. Wolfrom, M. W. Winkley, and S. Inouye, *Carbohydrate Res.*, 1969, **10**, 97.
[621] L. V. Fisher, W. W. Lee, and L. Goodman, *J. Heterocyclic Chem.*, 1969, **6**, 949.
[622] M. W. Winkley and R. K. Robins, *J. Org. Chem.*, 1969, **34**, 431.
[623] M. Jarman and W. C. J. Ross, *J. Chem. Soc.* (*C*), 1969, 199.
[624] J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 1969, **34**, 2646.
[625] F. W. Lichtenthaler, G. Trummlitz, and H. Zunke, *Tetrahedron Letters*, 1969, 1213.
[626] K. K. Gauri, K.-W. Pflughaupt, and R. Müller, *Z. Naturforsch.*, 1969, **24***b*, 833.
[627] A. F. Wu and E. Chargaff, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **63**, 1222.
[627a] G. Gough, M. H. Maguire, and F. Michal, *J. Medicin. Chem.*, 1969, **12**, 494.
[628] D. A. Shuman, A. Bloch, R. K. Robins, and M. J. Robins, *J. Medicin. Chem.*, 1969, **12**, 653.
[629] C. Cristescu, *Rev. Roumaine Chim.*, 1968, **13**, 365.
[630] G. T. Rogers and T. L. V. Ulbricht, *Chem. Comm.*, 1969, 508.
[631] G. T. Rogers and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1969, 2450.
[632] K. A. Watanabe and J. J. Fox, *J. Heterocyclic Chem.*, 1969, **6**, 109.
[633] H. Köster and G. Schramm, *Chem. Ber.*, 1969, **102**, 3868.



(439) R = OBz
(440) R = H



(441) R = OH
(442) R = H

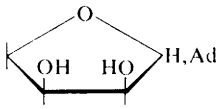


(443)

ester alone, caused elimination and afforded the hydroxy-glycal derivative (443) in high yield.

A disaccharide nucleoside, 9-[2′(3′)-*O*-D-ribosyl-β-D-ribofuranosyl]-adenine, has been synthesised by direct condensation of ribose and adenosine in the presence of hydrogen chloride.[634] Preliminary results have indicated that purine nucleosides can be produced from the thallium salts of the base.[635] These salts were stable, easily purified, and had good solubility properties. Substitution occurred primarily at position 9.

The reaction of 2,3-*O*-isopropylidene-β-L-erythrofuranosyl chloride with 6-benzamidopurine, followed by the removal of the protecting group, afforded [636] the α- and β-anomers of (444) in the ratio of 1 : 30. Thus, there



(444)

was almost complete retention of configuration in a system where there was no participating group, indicating that an S_N1 mechanism was involved.

6-(6-Amino-purinyl)-1,5-anhydro-6-deoxy-L-allitol, a nucleoside analogue with a methylene group between the sugar and the base, has been synthesised as illustrated [637] (Scheme 97).

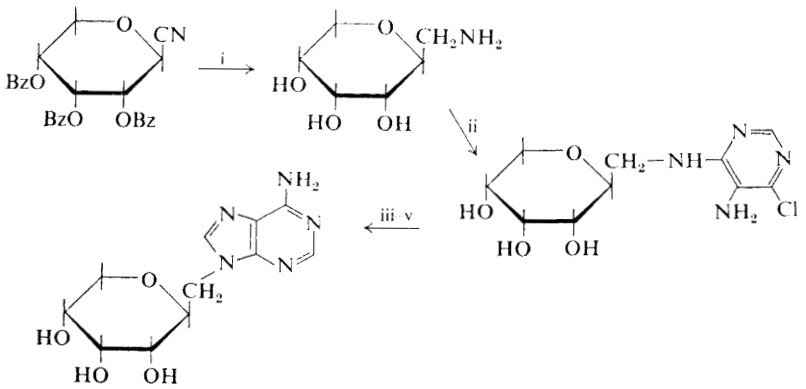
A series of nucleoside analogues, for example (445), of uracil, thymine, and cytosine have been prepared by reaction of (446) with the base in the presence of sodium iodide and potassium carbonate.[155]

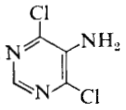
The homonucleosides (448) and (449) have been synthesised from (447) by reduction with LAH, reaction with nitrourea, treatment with β-ethoxyacryloyl chloride, and cyclisation, followed by the removal of blocking groups.[638]

Adenosine nucleosides containing a 5′-OSO_2NH_2 group have been synthesised as models for nucleocidin.[264]

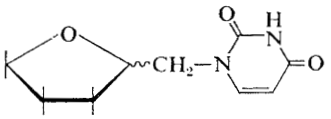
The racemic forms of the carbocyclic analogues of adenosine, inosine, 6-mercaptopurine ribonucleoside, and 6-(methylthio)purine ribonucleoside

[634] A. W. Lis and W. E. Passarge, *Physiol. Chem. Phys.*, 1969, **1**, 68.
[635] E. C. Taylor, Y. Maki, and A. McKillop, *J. Org. Chem.*, 1969, **34**, 1170.
[636] L. M. Lerner, *J. Org. Chem.*, 1969, **34**, 101.
[637] R. Vince and J. Donovan, *J. Medicin Chem.*, 1969, **12**, 175.
[638] M. Bobek and J. Farkaš, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1684.

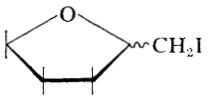


Reagents: i, LAH; ii,  ; iii, $HC(OEt)_3$; iv, NH_3; v, H^+

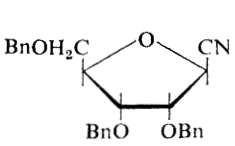
Scheme 97



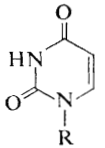
(445)



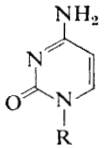
(446)



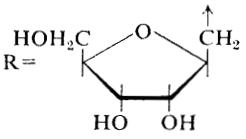
(447)

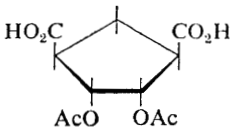


(448)

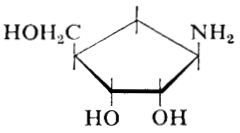


(449)

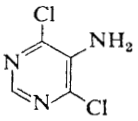




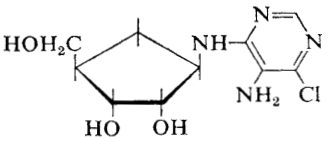
(450)



(451)



(452)



(453)

have been synthesised.[639] Compound (450) was converted into (451), condensation of which with (452) gave (453) from which the purine nucleosides were prepared. Racemic carbocyclic analogues of 2′-deoxy-adenosine and 3′-deoxy-adenosine have also been prepared.[640] Other carbocyclic pyrimidine nucleosides have been described.[641]

Bis(trimethylsilyl)-N^6-benzoyl-adenine and 2,3,5-tri-*O*-benzoyl-ribofuranosyl bromide have been allowed to react at room temperature in benzene, in the presence of mercuric bromide, to afford the 9-linked nucleoside (40% yield) and the 7-isomer (30% yield). The 7-isomer rearranged to the 9-isomer when heated with mercuric chloride in benzene.[642] The mercury derivative of 2,4-dihydroxy-5-ethoxycarbonyl-pyridine was condensed with tri-*O*-benzoyl-ribofuranosyl chloride to yield, after debenzoylation, a nucleoside which was a possible antagonist of pyridine nucleoside coenzymes.[643] *O*- and *N*-Glucosides were formed from reactions of acetobromoglucose with silver salts of uracil and thymine.[644]

Good yields of the corresponding β-glycosyl derivatives were obtained by the reaction of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide and 5,6-substituted-1-trimethylsilylbenzotriazoles.[645] The glycosylation of 6-azathymine by a silylation process has also been described.[646] Compound (454) was prepared by a standard condensation involving a trimethylsilylated enol.[647]

The oxidation of 2′,3′-*O*-isopropylidene-adenosine and 2-amino-2′,3′-*O*-isopropylidene-9-(β-D-ribofuranosyl)purine with alkaline permanganate solution gave the 5′-carboxylic acids.[548]

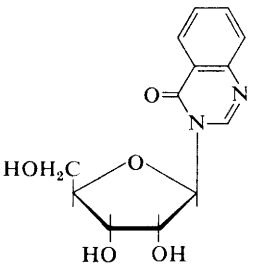
Esters

2′,3′-*O*-Phenylboronates of adenosine, uridine, inosine, and cytidine have been prepared;[648] the protecting group was removed under mild conditions with propane-1,3-diol. Other nucleoside phenylboronates [280] and other borate complexes [649] of adenosine and inosine have been described.

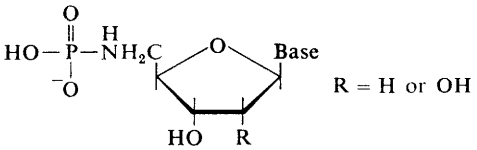
In order to elucidate the significance in relation to biological activity of the hydroxy-groups at C-3 and C-4 of the glucose portion of uridine-5′-(2-deoxy-α-D-*arabino*-hexopyranosyl pyrophosphate), the 3-deoxy- and the 4-deoxy-analogues have been synthesised.[650] The phosphorylation of

[639] Y. F. Shealy and J. D. Clayton, *J. Amer. Chem. Soc.*, 1969, **91**, 3075.
[640] Y. F. Shealy and C. A. O'Dell, *Tetrahedron Letters*, 1969, 2231.
[641] T. Suami, Y. Sato, Y. Fukai, and Y. Sakota, *J. Heterocyclic Chem.*, 1969, **6**, 663.
[642] B. Shimizu and A. Saito, *Agric. Biol. Chem. (Japan)*, 1969, **33**, 119.
[643] P. C. Jain and N. Anand, *Indian J. Chem.*, 1968, **6**, 767.
[644] G. T. Rogers, R. S. Shadbolt, and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1969, 203, 209.
[645] G. R. Revankar and L. B. Townsend, *J. Heterocyclic Chem.*, 1968, **5**, 785.
[646] M. Prystaš and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1104.
[647] M. G. Stout and R. K. Robins, *J. Heterocyclic Chem.*, 1969, **6**, 89.
[648] A. M. Yurkevich, I. I. Kolodkina, L. S. Varshavskaya, V. I. Borodulina Shvetz, I. P. Rudakova, and N. A. Preobrazhenskii, *Tetrahedron*, 1969, **25**, 477.
[649] T. Okano, T. Komatsu, T. Nara, and K. Tsuji, *J. Pharm. Soc. Japan*, 1969, **89**, 51.
[650] N. K. Kochetkov, E. I. Budowsky, T. N. Druzhinina, N. D. Gabrielyan, I. V. Komlev, Yu. Yu. Kusov, and V. N. Shibaev, *Carbohydrate Res.*, 1969, **10**, 152.

5′-deoxy-5′-aminonucleosides has been described. Conventional procedures were used to form the previously unknown *N*-5′-nucleoside phosphoramidates, for example (455), which were very unstable and hydrolysed even in



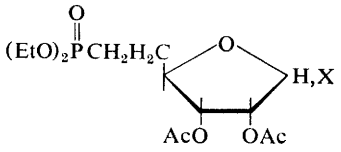
(454)



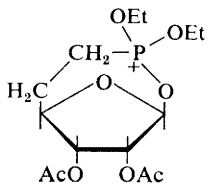
(455)

buffer solution.[651] The phosphorylation of *lyxo*-, *xylo*-, and *arabino*-furanosyl derivatives of uracil and thymine with triethyl phosphite has been studied.[652]

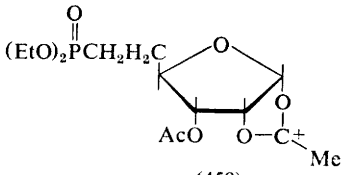
The attempted synthesis of purine nucleosides by the fusion or chloromercury salt methods with the ribofuranose derivatives (456) gave anomeric mixtures that had $\alpha:\beta$ ratios of 1 : 1 and 2 : 1 respectively.[653] This



(456)



(457)



(458)

unexpected result was believed to result from competition in participation between the diethyl-phosphinate group (457) and the 2-acetate group (458).

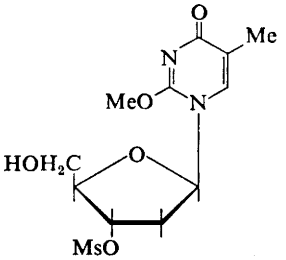
The β-benzoylpropionyl group has been described as a participating group in nucleotide and oligonucleotide synthesis.[225] It has the useful

[651] B. Jastorff and H. Hettler, *Tetrahedron Letters*, 1969, 2543.
[652] A. Holý and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1929.
[653] J. A. Montgomery and K. Hewson, *Chem. Comm.*, 1969, 15.

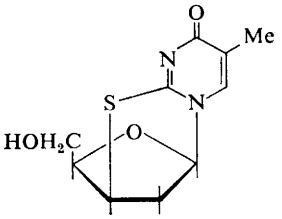
property of being easily removed in effectively neutral solutions of hydrazine hydrate in pyridine–acetic acid. 2′,3′-Dimethoxymethylidene ribonucleoside derivatives have been prepared and converted into cyclic carbonates by treatment with acid.[234a] Nucleoside 2′,3′ cyclic phosphates and 2′,3′ cyclic carbonates have been converted directly into anhydronucleosides.[654]

Other Nucleoside Derivatives

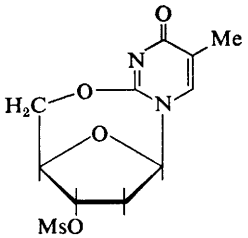
5′-Deoxy-5′-chloro(bromo)uridine has been prepared in high yield by reaction of uridine with the dimethyl halogenomethylene ammonium halides, $[(Me)_2N{=}CHCl]^+Cl^-(Br^-)$.[655] A series of thiosugar nucleoside anhydrides and related compounds containing sulphur in the anhydro-ring have been synthesised and investigated.[656] For example, the reaction of (459) with hydrogen sulphide in DMF afforded (460) which was converted



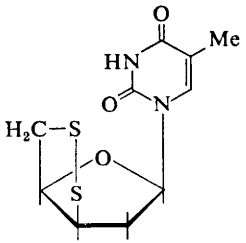
(459)



(460)



(461)



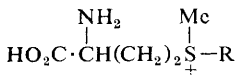
(462)

into a 3′-thionucleoside by alkali. The 3′-thionucleoside was also obtained by similar treatment of the 2,5′-anhydride (461). Compound (462), also prepared by an alternative route, was a by-product of this latter reaction.

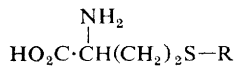
8-Bromonucleosides have been converted into anhydronucleosides by use of sodium hydride.[657]

[654] J. Nagyvary, *J. Amer. Chem. Soc.*, 1969, **91**, 5409.
[655] R. F. Dods and J. S. Roth, *Tetrahedron Letters*, 1969, 165.
[656] I. Wempen and J. J. Fox, *J. Org. Chem.*, 1969, **34**, 1020.
[657] P. C. Srivastava, K. L. Nagpal, and M. M. Dhar, *Indian J. Chem.*, 1969, **7**, 1.

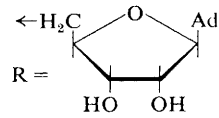
The glycosyl bond of *S*-adenosylmethionine (463) was found to be two orders of magnitude more stable in acid than that of *S*-adenosyl-homo-



(463)



(464)



cysteine [658] (464). This result indicated the importance of inductive effects of remote groups on reactions at anomeric centres.

Physical Measurements*

Mass spectral comparisons of the *C*-linked antibiotic nucleosides, formycin, formycin B, and showdomycin, with *N*-linked nucleosides have been reported,[155a] and the mass spectra of uridine and pseudouridine have also been discussed.[659] A structure for nucleocidin has been proposed on the basis of ^{19}F and ^{1}H n.m.r. and mass spectrometric evidence.[304]

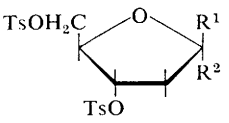
The crystal structures of a large number of nucleoside derivatives have been determined.[660–668] O.r.d. and c.d. studies of a variety of nucleosides have been reported [669–674a] and two particularly interesting applications of n.m.r. spectroscopy have appeared.[675, 676] Pyrolysis–gas chromatography has been used as a method for the characterisation of nucleosides and nucleotides.[676a]

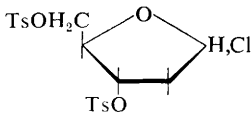
[658] F. Schlenk and C. R. Zydek-Cwick, *Arch. Biochem. Biophys.*, 1969, **134**, 414.
[659] J. M. Rice and G. O. Dudek, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 383.
[660] P. Tougard, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 961.
[661] P. Tollin and A. R. I. Munns, *Nature*, 1969, **222**, 1170.
[662] D. J. Hunt and E. Subramanian, *Acta Cryst.*, 1969, **25B**, 2144.
[663] D. W. Young, P. Tollin, and H. R. Wilson, *Acta Cryst.*, 1969, **25B**, 1423.
[664] C. E. Bugg and U. Thewalt, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 623.
[665] S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1969, **91**, 1210.
[666] W. Murayama, N. Nagashima, and Y. Shimizu, *Acta Cryst.*, 1969, **25B**, 2236.
[667] W. Saenger and F. Eckstein, *Angew. Chem. Internat. Edn.*, 1969, **8**, 595.
[668] E. Shefter, M. Barlow, R. A. Sparks, and K. N. Trueblood, *Acta Cryst.*, 1969, **25B**, 895.
[669] W. Voelter, G. Barth, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, 1969, **91**, 6165.
[670] T. Samejima, M. Kita, and M. Irie, *J. Biochem. (Japan)*, 1969, **65**, 305.
[671] T. Ueda and H. Nishino, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 920.
[672] D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, 1969, **91**, 824.
[673] D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, 1969, **91**, 831.
[674] D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **62**, 22.
[674a] T. Samejima, M. Kita, M. Saneyoshi, and F. Sawda, *Biochim. Biophys. Acta*, 1969, **179**, 1.
[675] J. H. Prestegard and S. I. Chan, *J. Amer. Chem. Soc.*, 1969, **91**, 2843.
[676] A. J. Jones, M. W. Winkley, and D. M. Grant, *Tetrahedron Letters*, 1969, 5197.
[676a] L. P. Turner, *Analyt. Biochem.*, 1969, **28**, 288.

* See also Chapters 23—25.

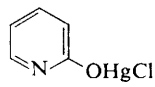
5-Chlorouridine crystallised with other nucleosides to form base-paired parallel-stranded ribbons.[677]

Several papers have commented on the adherence to, or deviation from, Hudson's Rules by various nucleoside derivatives. The anomeric pairs of 9-D-arabino- and 9-D-xylopyranosyladenines (together with smaller amounts of 7-α-D-arabino- and 7-β-D-xylopyranosyladenines as by-products) were prepared by standard reactions.[678] The 9-arabinosyl compounds disobeyed Hudson's Rule, whereas the 9-xylosyl analogues were in agreement with it. The products were examined in detail by n.m.r. methods, and the influence of different substituents on the glycosylating agent on the $\alpha:\beta$ ratio of the products was discussed. The 9-(6-mercaptopurine)nucleosides of α- and β-arabinopyranose and β-D-xylopyranose and 9-(β-D-xylopyranosyl)thioguanine have been prepared, and their structures assigned by n.m.r. methods.[679] Some of the pentopyranosylpurines which were described obeyed Hudson's Rule, whereas others did not. The structures of compounds (465) and (466), prepared from reaction of (467)

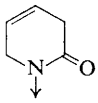


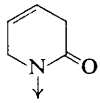


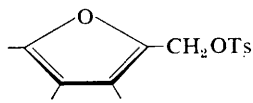
(467)



(468)

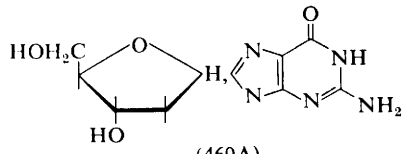
(465) R^1 =  , $R^2 = H$

(466) $R^1 = H$, R^2 = 



(469)

with (468), were assigned by n.m.r. methods;[680] the furan derivative (469) was produced in appreciable amounts as a by-product. Hudson's Rule was not obeyed by (465) and (466). The nucleosides (469A) were separated chromatographically into anomers which obeyed Hudson's Rule.[680a]



(469A)

[677] C. L. Coulter and S. W. Hawkinson, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **63**, 1359.
[678] A. P. Martinez, W. W. Lee, and L. Goodman, *J. Org. Chem.*, 1969, **34**, 92.
[679] A. P. Martinez and W. W. Lee, *J. Org. Chem.*, 1969, **34**, 416.
[680] U. Séquin and C. Tamm, *Helv. Chim. Acta*, 1969, **52**, 1219.
[680a] M. J. Robins and R. K. Robins, *J. Org. Chem.*, 1969, **34**, 2160.

22
Oxidation and Reduction

Periodate Oxidation

The structures and properties of the periodate oxidation products of glycosides and related derivatives have been reviewed.[681]

The periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-alloside and -β-D-mannoside in aqueous solution has been studied and the results compared with previous results on similar compounds with *altro*-, *gluco*-, and *manno*-configurations.[682] A detailed study has been made of the periodate oxidation of the six methyl amino-4,6-*O*-benzylidene-deoxy-α-D-glycopyranosides having *allo*-, *gluco*-, and *manno*-configurations.[683] Kinetic data were presented which showed that the rate-determining step in each reaction lay on a reaction path common to all the compounds. The order of rates was found to parallel the order of cuprammonium complexing, but it was stressed that too close a comparison between the two reactions should be avoided.

2-Methoxymalonaldehyde, which arises from the periodate oxidation of such molecules as 3-*O*-methyl-D-glucose, has been shown to react stoicheiometrically with periodate in 0·1N sulphuric acid at 0°, reducing two moles of periodate ion[684] (*cf.* vol. 1, p. 172). Under the usual conditions of periodate oxidation inositols give abnormal results and considerable over-oxidation occurs. However, it has now been shown that in 0·1N sulphuric acid at 0°, inositols consume exactly six molar equivalents of periodate.[685] Possible reasons for the difference were discussed.

DMSO-based Oxidations

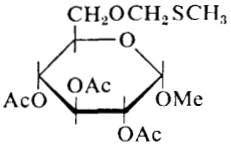
The use of DMSO-based oxidation mixtures in carbohydrate chemistry is now almost routine, and many of the ulose derivatives described in Chapter 16, or mentioned as precursors of branched-chain sugars in Chapter 15, were prepared by DMSO-based oxidation. One paper,[686] however, has described an extension of DMSO-type oxidations. A

[681] Z. Fialkiewiczowa, *Wiad. Chem.*, 1969, **22**, 421 (*Chem. Abs.*, 1969, **70**, 4444*r*).
[682] C. B. Barlow and R. D. Guthrie, *Carbohydrate Res.*, 1969, **11**, 565.
[683] C. B. Barlow and R. D. Guthrie, *Carbohydrate Res.*, 1969, **11**, 53.
[684] J. P. Girma, M.-T. Rokicka, and P. Szabó, *J. Chem. Soc.* (*C*), 1969, 909.
[685] S. R. Sarfati and P. Szabó, *Carbohydrate Res.*, 1969, **11**, 571.
[686] R. E. Harmon, C. V. Zenarosa, and S. K. Gupta, *Chem. Comm.*, 1969, 327.

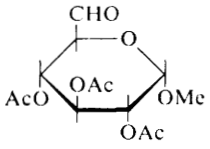
mixture of DMSO, phosphoric acid, and diphenylketen-*p*-tolylimine (470) oxidised 2′,3′-*O*-isopropylidene-adenosine to the corresponding 5′-aldehyde in 60% yield (as estimated from the 2,4-dinitrophenylhydrazone derivative).

$$Ph_2C{=}C{=}N{-}C_6H_4{-}Me(p)$$

(470)



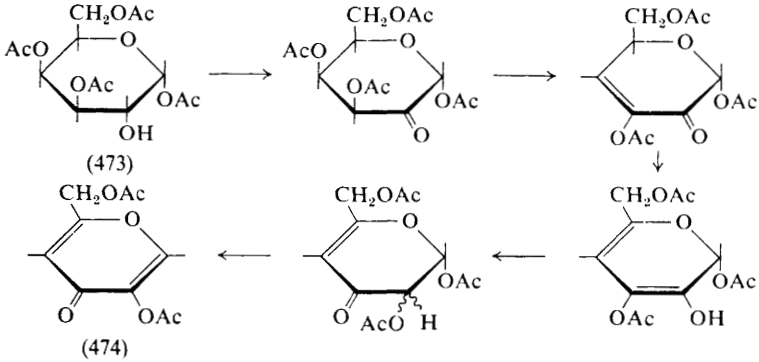
(471)



(472)

The reaction of methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside and acetic anhydride in DMSO yielded (471), whereas oxidation with the Pfitnzer–Moffatt reagent gave (472), identified as its *p*-nitrophenylhydrazine derivative.[686a]

Kojic acid diacetate (474) has been formed in high yield by treatment of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (473) and -α-D-glucopyranose with DMSO–acetic anhydride. The sequence of reactions shown in Scheme 98 was suggested, and the significance of the reactions discussed.[687]



(473)

(474)

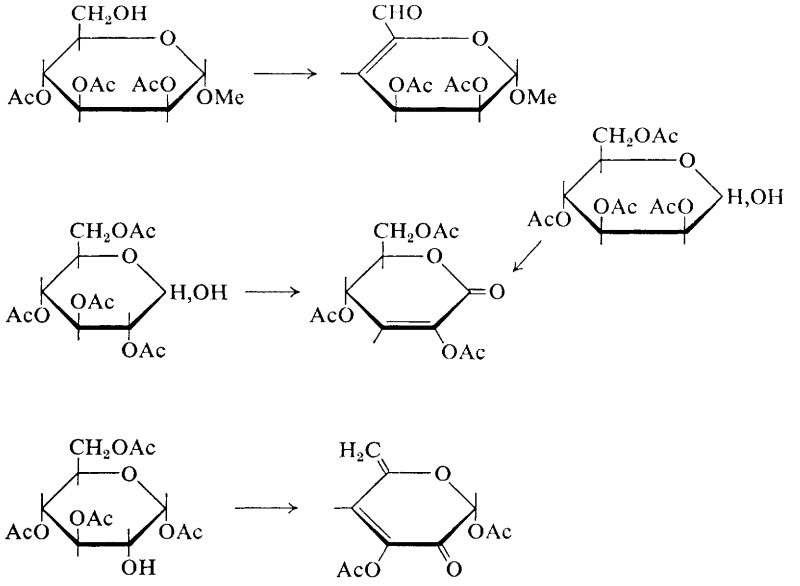
Scheme 98

The oxidation with DMSO, sulphur trioxide, pyridine, and triethylamine of variously protected carbohydrates containing one free hydroxy-group has been studied.[688] For acetylated compounds, elimination β to the derived carbonyl groups occurred, presumably under the catalytic influence of the base. Examples are shown in Scheme 99. Oxidations with this reagent of

[686a] B. A. Dmitriev, A. A. Kost, and N. K. Kochetkov, *Izvest. Akad. Nauk. S.S.S.R. Ser. khim.*, 1969, 697.

[687] G. J. F. Chittenden, *Carbohydrate Res.*, 1969, **11**, 424.

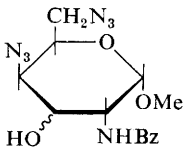
[688] G. M. Cree, D. W. Mackie, and A. S. Perlin, *Canad. J. Chem.*, 1969, **47**, 511.



Scheme 99

carbohydrates protected with isopropylidene, benzyl, or trimethylsilyl groups occurred smoothly, and work up was found to be more satisfactory than for other DMSO-based oxidations. Attempts to carry out intramolecular oxidations with a sulphoxide of 1-*S*-ethyl-1-thio-L-galactitol under a variety of conditions were unsuccessful.[415]

The attempted oxidation of either of the C-3 epimers (475) with acetic anhydride or benzoic anhydride in DMSO afforded only the acetate or benzoate in good yield.[268]



(475)

Other Oxidations

An improved procedure for the oxidation of carbohydrate derivatives with ruthenium tetraoxide (ruthenium dioxide/potassium periodate) has been described.[689] Potassium periodate was claimed to have solubility advantages over sodium periodate. It has been demonstrated that ruthenium tetraoxide is a satisfactory oxidant for free hydroxy-groups in glycosides

[689] B. T. Lawton, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1969, **10**, 456.

protected with benzoic and sulphonic acid esters and containing acetamido-groups.[519] Ruthenium tetraoxide also converts 1,4:3,6-dianhydro-hexitols into the corresponding 2,5-diones.[519] Like DMSO-based oxidants, ruthenium tetraoxide has found application in the preparation of many of the keto-sugars described in Chapters 15 and 16.

A mercuric oxide–iodine reagent in non-polar solvents has been shown to cleave vicinal diols to give aldehydic products, the yield being highest when the reaction was carried out in the dark.[690] This reagent cleaved *cis*- and *trans*-vicinal diols, and in cases such as *trans*-2,3-bornane-diol was effective, whereas periodate is not.[691] The reagent has not yet been used with carbohydrate derivatives. Another oxidising agent has been reported[692] that may have some application in carbohydrate chemistry; silver carbonate on Celite oxidised 1,2-, 1,3-, and 1,4-cyclohexanediols to hydroxy-ketones, whereas 1,4-, 1,5-, and 1,6-diols gave γ-, δ-, and ε-lactones.

Manganese dioxide has been used for the oxidation of hydroxy-groups situated α to double bonds.[509, 510] Chromium trioxide in pyridine has also been found to be satisfactory for oxidising this kind of compound.[455]

Chromium trioxide in acetic acid oxidised peracetylated methyl hexofuranosides and α-pyranosides to methyl 4-oxo- and 5-oxo-glyconates respectively;[520a] the 1-*O*-formyl derivative was formed when methyl tetra-*O*-acetyl-α-D-glucopyranoside was oxidised by chromium trioxide–acetic acid. Hot methanolic solutions of mercury(II) acetate have been used to oxidise D-arabinitol to various pentuloses.[508]

Reduction

The partial reduction of the δ-lactone of 4,6-dideoxy-L-*ribo*-hexonic acid to 4,6-dideoxy-L-*ribo*-hexopyranose has been studied with a number of complex hydrides.[445] The best results were obtained using LAH, which afforded the free sugar in 90% yield. Good yields of free sugars were also obtained on reduction of a number of lactones of aldonic acids with LAH in equimolar pyridine–tetrahydrofuran mixtures.[693]

[690] A. Goosen and H. A. H. Laue, *J. Chem. Soc.* (*C*), 1969, 383.
[691] A. Goosen and H. A. H. Laue, *J. Chem. Soc.* (*B*), 1969, 955.
[692] M. Fétizon, M. Golfier, and J.-M. Louis, *Chem Comm.*, 1969, 1102, 1118.
[693] J. Němec and J. Jarý, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1611.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

As in previous volumes, n.m.r. spectroscopy and stereochemical aspects of carbohydrate chemistry will be treated together. As an understanding of this branch of spectroscopy increases, its value in stereochemical analysis also rises, and this year ^{15}N, ^{31}P, and especially ^{13}C studies have indicated that appreciable developments must still be awaited. Too many n.m.r. results are being published for each to be mentioned individually, and only papers of the most general interest and value will be covered. There has been a notable increase in the application of n.m.r. spectroscopy to the study of acyclic carbohydrate derivatives, and a wider understanding is rapidly being gained of the preferred conformations of this class of compound in solution.

A most useful review has appeared covering all aspects of the application of n.m.r. spectroscopy to carbohydrates.[694] Another of significance to the subject, but not specifically related to it, dealt with the correlation of n.m.r. spin–spin coupling constants of protons with their geometrical relationships; a wide range of examples was given.[695] More specifically, a discussion has been published on the relationship between coupling constants and dihedral angles for the two protons of the >CH,OH system following observations on some sterol derivatives. The work provided the values $J = 0{\cdot}3$ and $10{\cdot}5$ Hz for the angles 80 and 160° respectively.[696]

It has been noted that vicinal vinylic coupling is much smaller for vinyl ethers (in particular glycals) than for related olefinic compounds.[455]

Pyranoid Systems

General Observations on Model Compounds.—The microwave spectrum of tetrahydropyran has been interpreted as indicating that the compound exists in the chair conformation,[697] and a mathematical method was described for calculating the barrier to interconversion of pyranoid ring conformations. It was determined that the energy required for converting

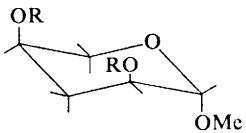
[694] T. D. Inch, *Ann. Rev. N.M.R. Spectroscopy*, 1969, **2**, 35.
[695] S. Sternhell, *Quart. Rev.*, 1969, **23**, 236.
[696] R. R. Fraser, M. Kaufman, P. Morand, and G. Govil, *Canad. J. Chem.*, 1969, **47**, 403.
[697] V. M. Rao and R. Kewley, *Canad. J. Chem.*, 1969, **47**, 1289.

the chair to a boat is 13·5 kcal mol^{-1}, and 6·5 kcal mol^{-1} for the alternative conversion.[698]

Dipole moments, Kerr constants, and refractivities of 2-chlorotetrahydropyran have been found to be in agreement with earlier n.m.r. results, and indicate that the molecule exists in the chair conformation with the chlorine atom axial.[699] In the course of work with the *cis*- and *trans*-isomers of 4-methoxytetrahydropyran-2-ol in aqueous 1,2-dimethoxyethane, it was found[474] that the anomeric effect has the value 0·9 kcal mol^{-1}, and the effect for the ethoxy-groups in 2,6-diethoxytetrahydropyran was found to be 2·3 (in ether), 1·9 (carbon tetrachloride), 1·4 (ethanol), and 1·1 kcal mol^{-1} (water).[700]

An important full paper on the effect of solvent on carbohydrate conformations and their optical rotations has been published from Lemieux's laboratory. A variety of deoxypyranosides including *S*(+)-2-methoxytetrahydropyran, were synthesised and examined in various solvents, and it was concluded that solvents capable of donating a hydrogen to form a hydrogen bond affect the anomeric effect more directly than through the general influence of dielectric constant change. Water weakened the anomeric effect specifically. This paper dealt with detailed solvation and conformational and optical rotational aspects of carbohydrates more fully than any previous publication.[701]

Another highly significant paper from the same laboratory has provided evidence that meaningful quantitative conformational analysis of molecules that contain interacting atoms having unshared electron pairs must consider the substituents on these atoms. Thus (476) exists in the illustrated



(476) R = Ac
(477) R = Me

conformation in non-polar solvents, whilst the ether (477) adopts the alternative chair form. This was interpreted in terms of interactions between axial oxygen atoms of different electron densities, there being less repulsion in the case of derivatives having electron-withdrawing *O*-substituents. Evidence was presented which indicated that hydrogen bonds between two hydrogen-bonded axial hydroxy-groups are strengthened by

[698] K. M. Grushetskii, *Zhur. strukt. Khim.*, 1968, **9**, 870.
[699] J. M. Eckert and R. J. W. Le Fèvre, *J. Chem. Soc.* (*B*), 1969, 855.
[700] A. Kankaanperä and K. Miikki, *Acta Chem. Scand.*, 1969, **23**, 1471.
[701] R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Canad. J. Chem.*, 1969, **47**, 4427.

bonding of the free group to the solvent. It was suggested that such hydrogen-bond conjugation may be important in biological systems.[702]

General Observations on Pyranoid Carbohydrates.—A review which gives the most up-to-date account of the conformations of aldoses in aqueous solution, and of the equilibria between the possible forms has been published by Angyal. The author's own work, which has contributed so appreciably to these subjects, was surveyed in particular.[45]

The n.m.r. spectra of a variety of acetylated monosaccharides have been studied in benzene and deuteriated chloroform. In all cases acetoxy-proton resonances occurred at higher fields in benzene (0·3—0·45 ppm), whereas the solvent shifts of ring protons were both positive and negative. The findings were discussed in terms of a benzene–solute association model.[703] In perfluorobenzene, the acetoxy-proton shifts were small and negative, whereas the ring proton shifts were large and positive. In pyridine, even larger ring proton shifts were observed.[704]

A full survey of the chemical shifts of acetoxy- and acetamido-group resonances in peracetylated amino-sugars and nucleosides has been reported. The acetoxy-group signals were not affected by a solvent change from deuteriated chloroform to DMSO, but acetamido-groups underwent a diamagnetic shift of 0·15 ppm. In the peracetylated hexopyranosyl nucleosides, the anisotropy of the bases caused a general diamagnetic shift of the C-2′ acetoxy-signals: 0·1 ppm in the case of pyrimidine compounds and 0·3 ppm for purine derivatives. Configurational analysis of nucleosides can be carried out on the basis of the observations made.[705]

The application of n.m.r. spectroscopy to the determination of the anomeric configurations of sugars liberated by glycosidases has been referred to in Chapter 3.

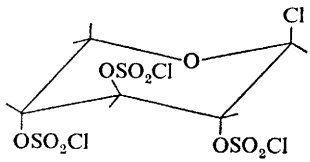
Specific Pyranoid Compounds.—Molecular orbital calculations have been proposed as an important method for the conformational analysis of biologically significant compounds. As an example, α-D-glucopyranose was shown, by application of an extended Huckel calculation, to adopt the *C*1 conformation.[706]

A thorough n.m.r. investigation has been reported of the conformational equilibria for the eight tetra-*O*-acetyl-D-pentopyranoses. Conformations were determined by the spin-coupling averaging method, and ΔG° values were recorded. Whereas the α-D-xylopyranose compound was shown to exist exclusively in the *C*1 conformation at 25°, the *β-ribo*-isomer (vol. 1, p. 52) and the *β*-D-*lyxo*-compound were conformational mixtures which were resolved or 'frozen out' at −97°. The polarity of the solvents used had little effect on the positions of the conformational equilibria.

[702] R. U. Lemieux and A. A. Pavia, *Canad. J. Chem.*, 1969, **47**, 4441.
[703] M. H. Freemantle and W. G. Overend, *J. Chem. Soc.* (*B*), 1969, 547.
[704] M. H. Freemantle and W. G. Overend, *J. Chem. Soc.* (*B*), 1969, 551.
[705] F. W. Lichtenthaler, G. Bambach, and U. Scheidegger, *Chem. Ber.*, 1969, **102**, 986.
[706] W. Brock Neely, *J. Medicin. Chem.*, 1969, **12**, 16.

Anomerisations were then carried out, and the $\alpha : \beta$-equilibrium constants were also recorded.[707] More detailed reports were also published on the conformational equilibria of β-D-ribopyranose tetra-acetate and the β-D-*xylo*-isomer. The various methods available for measuring conformational equilibria were critically compared, and the discussion in this paper constitutes an admirable review of the application of n.m.r. methods to conformational equilibria problems. Results were presented which showed that the method of averaging chemical shifts is unreliable for determining conformational equilibria. By the method of averaging of spin couplings, the former acetate was shown to exist at room temperature in acetone as a 9 : 11 mixture of 1*C* and *C*1 conformations, and the latter is a 1 : 5 mixture of these forms.[708]

The conformations adopted by the anomeric xylopyranosyl chloride 2,3,4-tri(chlorosulphates) and the lyxosyl analogues were those in which the glycosyl chlorine atoms were axial. The β-*xylo*-isomer (478), therefore,



(478)

provided another example of a pyranoid compound having all the ring substituents axial.[290]

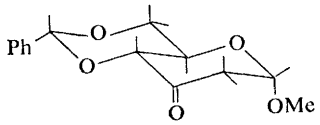
The n.m.r. spectra of some 1,6-anhydro-β-D-hexopyranoses have been reported. Long range 1,6- and 4,6-proton couplings were detected besides the expected 1,3-(eq,eq) couplings. Comparison between the spectra of these compounds and those of the corresponding 1,6-anhydro-β-D-hexopyranosuloses permitted the shifts induced by the anisotropic effects of the carbonyl groups to be determined.[708a] More specifically, the conformations of 1,6-anhydro-β-D-glucopyranose were studied mainly by i.r. spectroscopy. It was concluded that the compound adopts the 1*C* conformation when crystallised from solution, but on heating, or when it is crystallised from a melt, a boat ring shape is assumed.[184]

The study of selective substitution of polyhydroxy-compounds has received appreciable help from n.m.r. studies, since it has been possible to recognise individual substituents from the chemical shift of their resonances. Studies with acetates are described in Chapter 6 and with methyl ethers in Chapter 4.

[707] P. L. Durette and D. Horton, *Chem. Comm.*, 1969, 516.
[708] P. L. Durette, D. Horton, and N. S. Bhacca, *Carbohydrate Res.*, 1969, **10**, 565.
[708a] K. Heyns and J. Weyer, *Annalen*, 1968, **718**, 224.

A detailed study has been made of methyl 2,3- and 3,4-anhydro-α-D-glycopyranosides; all the compounds examined were found to adopt half-chair ring shapes. The predominant factor in determining which of the two possible half-chair conformations were favoured was the anomeric effect. Only in one case, in which there was a strong interaction between the epoxide ring and the aglycon, did the form having the latter group equatorial predominate.[709] The n.m.r. spectra of methyl 4,6-di-*O*-acetyl-2,3-anhydro-α-D-allo-, -manno-, -talo-, and -gulo-pyranosides have been recorded, and readily allow characterisation of the isomers.[451] Another compound which does not adopt a chair conformation is methyl 4,6-*O*-benzylidene-2-deoxy-2-*C*-pentachlorophenyl-α-D-altropyranoside, presumably because such a ring shape would require the bulky aryl group to be axial.[460a]

Several carbohydrate acetal derivatives have been examined. The spectra of the eight stereoisomers of methyl 2(and 3)-amino-4,6-*O*-benzylidene-2-(and 3)-deoxy-α-D-*erythro*-hexopyranosides were recorded and discussed; chemical shifts and coupling constants were consistent with expectations based on reported work.[710] It was ascertained by use of specifically deuteriated compounds that the axial C-2 proton in compound (479) resonates at lower fields than the equatorial proton.[520] The same observation was made for related compounds.[313]



(479)

A reference has been made to an effect of branch substituents on the chemical shifts of adjacent isopropylidene methyl signals.[493a]

The effects on the pyranoid ring shapes of sorbose and fructose of fused isopropylidene rings have been examined,[206] and n.m.r. spectroscopy has been used to assign the structure of 2,3:4,5-di-*O*-isopropylidene-β-D-fructose.[208]

The 100 and 220 MHz n.m.r. spectra of sucrose octa-acetate, 1-kestose hendeca-acetate, and nystose tetradeca-acetate (a homologous series containing 1, 2, and 3 fructofuranosyl residues) were measured in benzene, chloroform, and acetone. Signals for H-1, H-2, H-3, and H-4 of the α-D-glucopyranosyl residue and H-3 and H-4 of the β-D-fructofuranosyl residues could be observed individually for each of the compounds, and the data confirmed that all the fructose rings are furanoid. The 2–1

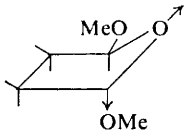
[709] J. G. Buchanan, R. Fletcher, K. Parry, and W. A. Thomas, *J. Chem. Soc.* (*B*), 1969, 377.

[710] C. B. Barlow, E. O. Bishop, P. R. Carey, and R. D. Guthrie, *Carbohydrate Res.*, 1969, **9**, 99.

linkages between the fructofuranosyl residues of kestose and nystose were also confirmed.[711]

Furanoid Systems

In equilibration studies of *cis*- and *trans*-2,5-dimethoxytetrahydrofuran, variations were noted in the ratio of the components with solvent, which were consistent with the operation of an anomeric effect. The *trans*-isomer was the more stable in non-polar solvents, in contrast to findings with 2,4-substituted-1,3-dioxolanes; the dipolar effect represented in (480) was believed to be responsible.[700]



(480)

^{13}C Studies have permitted distinctions to be made between nucleoside structures which were not possible by proton methods (see below). The conformations of uridine, its 3′- and 5′-phosphates, and its 2′-deoxy-derivative have been studied by p.m.r.; additions of 'structure-breaking' salts, *e.g.* magnesium perchlorate, caused conformational changes in the molecules, the puckering of the furanose ring, and the orientation of the uracil with respect to the sugar being affected.[675]

The detailed n.m.r. spectra (100 and 220 MHz) of *N*-acetyl-β-D-galacto-furanosylamine and α-D-glucofuranosylamine have been reported.[232]

Acyclic Systems

In this section genuinely acyclic molecules are considered together with cyclic derivatives of acyclic carbohydrates.

N.m.r. studies of the peracetylated diethyl dithioacetals of D-arabinose, D-lyxose, L-rhamnose, and L-fucose indicated that extended zig-zag conformations are adopted by these compounds in solution. Further, the D-ribose and D-xylose compounds have eclipsed acetoxy-groups at C-2 and C-4 in this conformation, and the spectra indicated that the resulting strain is relieved by rotation about the C-3,C-4 and C-2,C-3 bonds, respectively.[712] In related fashion, the vicinal coupling constants for the 3,4,5,6-tetra-acetyoxy-*trans*-1-nitro-1-hexenes indicated that the D-*xylo*- and D-*ribo*-isomers do not adopt a regular zig-zag shape, whereas the D-*arabino* compound does.[713] Other related work has examined the conformation of

[711] W. W. Binkley, D. Horton, and N. S. Bhacca, *Carbohydrate Res.*, 1969, **10**, 245.
[712] D. Horton and J. D. Wander, *Carbohydrate Res.*, 1969, **10**, 279.
[713] J. M. Williams, *Carbohydrate Res.*, 1969, **11**, 437.

an extensive series of acyclic compounds, and also has led to the conclusion that 1,3-eclipsed interactions, rather than 1,2-*gauche* interactions, are most important in determining the preferred conformations of acyclic carbohydrate derivatives.[714]

The 100 MHz n.m.r. spectra of 1:4,3:6-dianhydro-D-glucitol, -L-iditol, and -D-mannitol and their esters have been fully analysed, and interpreted as indicating that the five-membered rings have one conformation which is closely related to an envelope shape. It was noted that intramolecular hydrogen bonding which occurs in the D-glucitol and D-mannitol derivatives has little effect on the ring shapes.[715] The investigation (in thionyl chloride at temperatures down to $-82°$) of 2,5-*O*-methylene-D-mannitol derivatives having methylene or benzylidene acetals bridging the 1,3- and 4,6-positions has shown that the methylene protons of the seven-membered rings are magnetically equivalent. This was interpreted by assuming the 1,3-dioxepan rings to be in twist conformations, which put these protons in identical environments, rather than a fast ring-inversion process.[716]

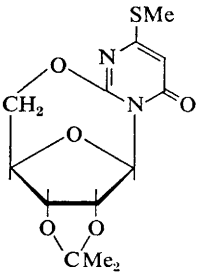
Heteronuclear N.M.R. Studies

Further detailed studies (*cf.* vol. 2, p. 197) have been undertaken of the 100 and 220 MHz proton and ^{19}F spectra of seventeen hexopyranosyl fluorides and their derivatives. H–F Couplings showed the same types of angular dependencies as H–H couplings, but were generally larger and could thus be used as more sensitive conformational probes. The following general values were noted for vicinal $^{1}H,^{19}F$-coupling constants: $J_{F_a,H_{2e}}$ 1—1·5 Hz, $J_{F_e,H_{2a}}$ 7·6—12·6 Hz, and $J_{F_a,H_{2a}}$ 24 Hz. Geminal couplings were 53·5 or 49 Hz depending upon whether the C-2 group was equatorial or axial.[716a] Thirteen pentopyranosyl fluorides were then similarly examined, and all were found to adopt conformations with the fluorine atoms in the axial orientation. Long-range couplings over four bonds were used to corroborate the findings.[717] Hall and Manville have also used 'selective-irradiation' and 'spin-tickling' techniques to determine the relative signs of the vicinal and geminal ^{19}F–^{1}H couplings in a series of glycosyl fluorides.[718]

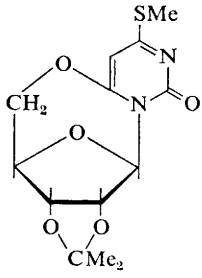
Kochetkov's group have used ^{19}F n.m.r. for quite a different purpose. They have found that the fluorine resonances of trifluoroacetyl esters show a chemical shift range which is 1·5—3 times greater than that shown by the proton resonances of acetates, and they demonstrated that these resonances are sufficiently characteristic to serve as a means of identifying esterified glycosides.[719]

[714] J. B. Lee and B. F. Scanlon, *Tetrahedron*, 1969, **25**, 3413.
[715] F. J. Hopton and G. H. S. Thomas, *Canad. J. Chem.*, 1969, **47**, 2395.
[716] T. B. Grindley, J. F. Stoddart, and W. A. Szarek, *J. Chem. Soc.* (*B*), 1969, 172.
[716a] L. D. Hall, J. F. Manville, and N. S. Bhacca, *Canad. J. Chem.*, 1969, **47**, 1.
[717] L. D. Hall and J. F. Manville, *Canad. J. Chem.*, 1969, **47**, 19.
[718] L. D. Hall and J. F. Manville, *Carbohydrate Res.*, 1969, **9**, 11.
[719] B. A. Dmitriev, A. V. Kessenich, A. Ya. Chernyak, A. D. Naumov, and N. K. Kochetkov, *Carbohydrate Res.*, 1969, **11**, 289.

^{13}C Magnetic resonance studies using the isotope in its natural abundance have shown that the technique offers appreciable promise for conformational studies of pyranoid compounds. Monoalkylcyclohexanols and their methyl ethers and their acetates, all showed that the chemical shifts of the ring carbons, which bore oxygenated functions, depended largely on their orientation. Atoms bearing equatorial groups resonated about 5 ppm upfield relative to those bearing the same groups in the axial orientation.[720] Hall and Johnson have applied the technique to carbohydrates and have shown it to be of considerable potential value. They found that the C-1 chemical shifts of pyranoid compounds depended upon the nature of the C-1 substituents and on the configuration of other carbon atoms. The shifts between anomers was independent of the nature of the C-1 substituent, and for D-glucose derivatives was $3{\cdot}7 \pm 0{\cdot}2$ ppm. Chemical shifts, relative to $^{13}CS_2$, of many of the carbon atoms encountered in carbohydrate derivatives were given.[721] Preliminary studies have been reported of the ^{13}C and ^{1}H spectra of D-glucose enriched with ^{13}C. The spectra of glucose and the enriched sugar were compared, and decoupling experiments were reported.[722] ^{13}C Resonance studies have enabled a distinction to be made between the anhydronucleosides (481) and (482).[676]



(481)



(482)

Derivatives of 6-amino-6-deoxy-D-glucose-6-^{15}N have been prepared, and their n.m.r. spectra examined. The nitrogen atom showed small coupling with H-5 and H-6, and 91·3 Hz coupling with the bonded hydrogen atom.[319] ^{31}P–^{1}H Couplings were studied during the investigation of carbohydrate *C*-phosphonates.[560]

[720] G. W. Buchanan and J. B. Stothers, *Canad. J. Chem.*, 1969, **47**, 3605.
[721] L. D. Hall and L. F. Johnson, *Chem. Comm.*, 1969, 509.
[722] A. S. Perlin and B. Casu, *Tetrahedron Letters*, 1969, 2921.

24
Other Physical Methods

Most notable in this area is the increasing status of mass spectrometry as an analytical tool in carbohydrate chemistry, and the rapid rate of growth in the presentation of *X*-ray crystallographic results.

I.r. Spectroscopy

An important review relating to all aspects of the infrared spectroscopy of carbohydrates has been written. The absorption of all relevant functional groups were considered, conformational applications were reported, and the use of the technique in analytical work was discussed.[723] A study of the i.r. absorptions of a large number of carbohydrates at low temperatures (down to 20 K) has shown that spectra of much better quality and of greater potential value are so obtained. Spectral changes with temperature were much greater than those usually observed with simple compounds. It was suggested that the technique may have application to the identification, characterisation, and differentiation of complex compounds of biological interest (*cf.* vol. 2, p. 198).[724]

Mass Spectrometry

Field ionisation mass spectra of free sugars have been studied, and the method shown to be excellent for revealing the M^+ and $(M+1)^+$ ions of mono- and di-saccharides. It thus is of particular importance as a means of determining accurate molecular weights of substituted and unsubstituted carbohydrate compounds.[43]

All other reports have related to electron bombardment spectra. The mass spectra of a series of trimethylsilyl derivatives of free sugars and glycosides have been examined, and fragmentation patterns were elucidated with the aid of deuterium labelling and exact mass studies. In conjunction with g.l.c., mass spectrometry was then utilised to examine the products of trimethylsilylation of free sugars and glycoside mixtures. Furanosyl derivatives were encountered in all cases. As has been previously shown, mass spectrometry provides powerful means of allocating ring sizes to trace components of mixtures.[176] The furanose nature of di-*O*-isopropylidene-D-talose derivatives has been established by this method.[203]

[723] R. S. Tipson, *National Bureau of Standards Monograph*, No. 110, June 1968.
[724] J. E. Katon, J. T. Miller, and F. F. Bentley, *Carbohydrate Res.*, 1969, **10**, 505.

The spectra of the phenylosazones of several monosaccharides were examined and fragmentation patterns were presented. All the compounds showed molecular ions.[367] Other nitrogenous compounds examined have been derivatives of pyranose and furanose 2-methyl- and 2-phenyl-glyc-(1′,2′:4,5)-2-oxazolines. Again the technique allowed the glycosyl ring size to be determined.[725] Spectra of several 4,6-*O*-benzylidene derivatives of 2-acetamido-2-deoxy-sugars have also been recorded.[353]

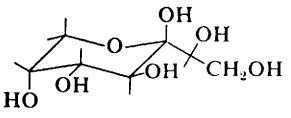
The spectra of some unsubstituted glycuronic acids and the 3,6-lactone of D-glucuronic acid have been reported. It was concluded that free D-glucuronic acid exists in the furanoid ring form, since it formed the furanoid lactone in the spectrometer, and since its spectrum was different from that of D-galacturonic acid.[726] Isopropylidene acetals of several uronic acids and their derivatives have also been examined.[551]

Differences in the mass spectra of dianhydrohexitols and their di-*O*-benzoyl esters reflect differences in their stereochemistry, and sometimes may be used to identify stereoisomers.[727]

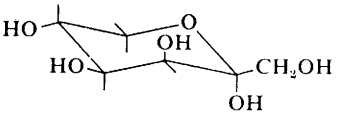
X-Ray Crystallography

Once again the number of reported crystallographic studies has increased, and nucleosides and related compounds have again attracted particular attention.

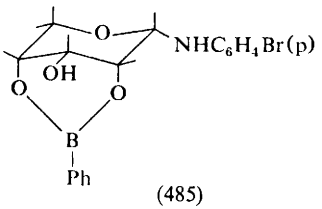
D-*manno*-3-Heptulose has been examined as its monohydrate and shown to be the β-pyranoid compound in the 1*C* conformation (483).[728] α-D-Tagatopyranose has the *C*1 ring form (484).[729]



(483)



(484)

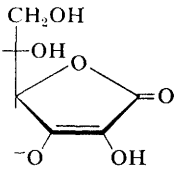


(485)

[725] N. S. Wulfson, V. N. Bochkarev, G. M. Zolotareva, T. R. Rajagopolan, A. J. Khorlin, I. M. Privalova, and M. L. Shulman, *Carbohydrate Res.*, 1969, **10**, 351.
[726] V. Kováčik and Š. Bauer, *Coll. Czech. Chem. Comm.*, 1969, **34**, 326.
[727] N. S. Wulfson, O. S. Chizhov, and L. S. Golovkina, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 768.
[728] T. Taga and K. Osaki, *Tetrahedron Letters*, 1969, 4433.
[729] S. Takagi and R. D. Rosenstein, *Carbohydrate Res.*, 1969, **11**, 156.

The 2,4-phenylboronate (485) has been shown to have the illustrated structure and conformation,[281] and the major product formed on treatment of tri-*O*-acetyl-D-glucal with 'BrF' has been shown to be the 2-bromo-2-deoxy-α-D-mannopyranosyl fluoride.[285] Other halogenated derivatives examined have been methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside, and both anomers of methyl 2-chloro-2-deoxy-D-galactopyranoside which co-crystallised as a liquid crystal. All these compounds adopted the *C*1 conformation.[730]

Sodium ascorbate exists in the crystal in the lactonised form with the ion having a structure intermediate between (486) and (487).[731] D-Glucono-1,5-lactone exists in the flattened-chair conformation (488).[526]



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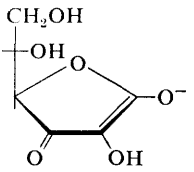

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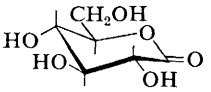
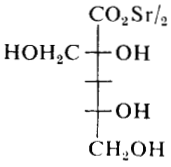


(488)

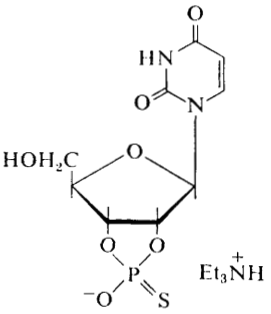
Continued interest has been shown in acyclic carbohydrates, and the results being obtained are correlating well with those derived from n.m.r. studies (p. 175). Ribitol crystallised as a racemate of right-handed and left-handed non-planar forms in which the O-2 and O-4 *cis*-interactions caused deviation from a symmetrical structure.[732] A similar situation exists with xylitol, and again the molecule exists in a non-planar conformation.[733] *X*-Ray analysis has shown that strontium D-*gluco*-isosaccharinate, obtained by treatment of 4-*O*-substituted D-glucoses with strontium hydroxide, has the *erythro*-structure (489).[495]

The following nucleosides have been examined (the furanosyl rings are all non-planar and the out-of-plane atoms are indicated in parentheses): 5-chlorouridine (2′) (this compound crystallises with the molecules forming a base-paired parallel stranded ribbon; it was suggested that this type of

[730] R. Hoge and J. Trotter, *J. Chem. Soc.* (*A*), 1969, 2165, 2170.
[731] J. Hvoslef, *Acta Cryst.*, 1969, **25B**, 2214.
[732] H. S. Kim, G. A. Jeffrey, and R. D. Rosenstein, *Acta Cryst.*, 1969, **25B**, 2223.
[733] H. S. Kim and G. A. Jeffrey, *Acta Cryst.*, 1969, **25B**, 2607.



(489)



(490)

structure might also be adopted by some polynucleotides),[677] 5-methyluridine (3′),[662] 3-(β-arabinofuranosyl)-5-bromouracil,[660] 8-bromoguanosine,[664] thymidine (3′),[663] and inosine (3′).[661]

Structures of nucleoside phosphate derivatives have been reported as follows: sodium inosine 5-phosphate (2′; the sodium ions were bound to the hydroxy-groups rather than to the phosphate anions),[665] guanosine 5-phosphate (3′),[666] compound (490) (ribose ring almost planar),[667] and the dinucleotide phosphate β-adenosine-2′-β-uridine 5′-phosphoric acid.[668]

Electron Spin Resonance Spectroscopy

The e.s.r. spectrum of single crystals of 2-deoxy-D-*erythro*-pentose after irradiation with *X*-rays has been reported and discussed; several radicals were detected.[438, 733a] Related studies on various hexoses [734] and several derivatives [735] have been described. Radicals generated by irradiation of frozen solutions of D-ribose [52] and various disaccharides [479] have also been investigated.[131]

[733a] J. Huttermann and A. Mueller, *Radiation Res.*, 1969, **38**, 248.
[734] H. Neubacher, *Z. Naturforsch.*, 1969, **24b**, 1184.
[735] N. V. Zakatova, D. R. Minkhadzhiddinova, and V. A. Sharpatyi, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1969, 1633.

25
Polarimetry

All the significant 1969 publications on polarimetry that have been located have been concerned with optical rotatory dispersion and circular dichroism.

The o.r.d., c.d., and u.v. spectra of *o*- and *p*-nitrophenyl α- and β-hexopyranosides have been measured. It was concluded that *o*-nitro-groups are twisted from the plane of the aromatic rings and so themselves become optically active chromophores giving Cotton effects near 340 nm. This accounts for the anomalous optical rotations of some *o*-nitrophenyl glycosides.[140] In related work, the relationship between the configurations at C-1 and the signs of the multiple Cotton effects observed near 260 nm in the o.r.d. spectra of phenyl glycosides were discussed.[736]

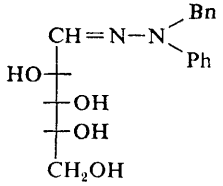
The chirality of *cis*- and *trans*-glycols can be determined by observation of the o.r.d. and c.d. curves of the derived dibenzoates. The signs of the Cotton effects near 233 nm correlate with the absolute configurations, and the effects arise from $\pi \rightarrow \pi^*$ transitions due to dipole interactions between the ester groups.[737] Chiralities of α-diols may also be determined from the signs and amplitudes of Cotton effects of cyclic thionocarbonates.[738] The circular dichroism of nitrites of 1,4:3,6-dianhydrohexitols has been examined,[739] as have those of molybdate complexes of aldonic acids,[523] free sugars,[44] and alditols.[740] With the last two sets of compounds, various diol configurations gave characteristic c.d. curves. The o.r.d. of the complexes formed between glucuronic acid,[550] or aldotetronolactones [522] and molybdic acid have also been reported.

The c.d. spectra of a number of uronic acids have been examined in aqueous solution. D-Hexuronic acids with equatorial hydroxy-groups at C-4 gave negative bands near 234 nm and positive bands near 206 nm. Only a positive band near 210 nm was observed for D-galacturonic acid and its derivatives.[741]

The absolute configurations of optically active 2,3-dihydroxybutyric acids (4-deoxy-erythronic and -threonic acids) have been determined by o.r.d. methods.[524]

[736] T. Sticzay, C. Peciar, and Š. Bauer, *Tetrahedron*, 1969, **25**, 3521.
[737] N. Harada and K. Nakanishi, *J. Amer. Chem. Soc.*, 1969, **91**, 3989.
[738] A. H. Haines and C. S. P. Jenkins, *Chem. Comm.*, 1969, 350.
[739] L. D. Hayward and R. N. Totty, *Chem. Comm.*, 1969, 997.
[740] W. Voelter, E. Bayer, R. Records, E. Bunnenberg, and C. Djerassi, *Chem. Ber.*, 1969, **102**, 1005.
[741] I. Listowsky, S. Englard, and G. Avigad, *Biochemistry*, 1969, **8**, 1781.

The o.r.d. spectra of several aldose benzylphenylhydrazones which exist in solution in the acyclic, rather than the glycosylamine, form revealed that compounds having the *R*-configuration at C-2 (*e.g.* 491) showed positive



(491)

Cotton effects near 300 nm and were dextrorotatory at high wavelengths. The opposite effects were noted for the C-2 epimers. Influences of *O*-acetylation were profound, in the majority of cases causing reversal of the sign of the rotations.[366] It was not found possible to predict the sign of the Cotton effects of a number of 4-azido-4-deoxypentopyranosides from the azide octant rule; however, some empirical observations were of interest. Those compounds with the *C*1(D) conformation having axial azido substituents exhibited positive Cotton effects, whereas the opposite held with equatorial azides; the situation was reversed for compounds in the 1*C*(D) ring form.[742]

O.r.d. and c.d. studies of immunochemically active oligo- and polysaccharides containing amino-sugars have been reported, and tentative rules have been proposed which may allow the nature and position of substitution on 2-acetamido-2-deoxy-D-glucose residues to be determined.[743]

A considerable body of new information has been gathered on the o.r.d. and c.d. of nucleosides and derivatives; in particular, the collaboration between physical and organic chemists at the University of Utah has proved fruitful. Four optically active transitions have been located and assigned in the c.d. spectra of sixteen uracil derivatives.[672] Similar results were obtained for twelve cytosine derivatives, and a method for determining the conformations of various cytidine and uridine nucleosides was proposed.[673] The same group of workers have also shown that the 260 and 207 nm absorptions of adenine compounds each derive from at least two electronic transitions.[674]

Other workers have studied the o.r.d. and c.d. curves obtained from sulphur-containing nucleosides,[674a] 2-thiopyrimidine nucleosides,[671] and 5,6-dihydrouridine phosphate.[670]

The magnetic c.d. spectra of some 3,5′-purine cyclonucleosides and of related bases have been recorded and compared with the spectra of the parent nucleosides. The results showed that the applicability of the method was the same as that of the simple c.d. of optically active compounds.[669]

[742] T. Sticzay, P. Šipoš, and Š. Bauer, *Carbohydrate Res.*, 1969, **10**, 469.
[743] E. V. Kabat, K. O. Lloyd, and S. Beychok, *Biochemistry*, 1969, **8**, 747.

26
Separatory and Analytical Methods

The policy adopted for this section follows that used in the previous volumes; no extensive cross-referencing to previous chapters is provided.

The continuing effort put into the development of new techniques, and the adaptation of older methods, seems surprising in view of the wealth of available information on some of these subjects.

Chromatographic Methods

Gas–Liquid Chromatography.—A g.l.c. method for the direct determination of alditols (up to the pentitols) has been applied to different biological systems. For example, the oxidation of D-arabinitol to D-*threo*-pentulose can be followed by removing the responsible bacterial cells and injecting the residual solutions directly on to columns at 150—250°C.[744] Another direct method is pyrolysis–g.l.c., which has been applied to the characterisation of nucleosides and nucleotides.[745]

Alditol acetates continue to be used as derivatives suitable for the analysis of free sugars. Conditions have been described for optimal separations of hexose, pentose, and deoxyaldose compounds.[746] Because of their value in current polysaccharide analytical work, the g.l.c. retention characteristics of a large range of acetylated glucitol methyl ethers and methyl glycoside methyl ethers have been reported.[747] Methyl ethers of 2-amino-2-deoxy-D-glucose have also been examined by this method.[166] For related reasons, the retention times of acetates, trimethylsilyl ethers, and trifluoroacetates of forty-one glycosides have been considered in relation to their structures.[748] The trifluoroacetates of alditols have received further recommendation as derivatives suitable for g.l.c.[226, 749]

Dimethylsilyl ethers of cyclodextrins can be used for analytical purposes on columns run between 325 and 400°C.[750]

Much consideration continues to be given to trimethylsilyl ethers, and these are now being utilised on a wide scale. The electron-capture detector

[744] L. Dooms, D. Declerck, and H. Verachtert, *J. Chromatog.*, 1969, **42**, 349.
[745] L. P. Turner, *Analyt. Biochem.*, 1969, **28**, 288.
[746] D. H. Shaw and G. W. Moss, *J. Chromatog.*, 1969, **41**, 350.
[747] H. G. Jones and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 3269.
[748] K. Yoshida, N. Honda, N. Iino, and K. Kato, *Carbohydrate Res.*, 1969, **10**, 333.
[749] T. Imanari, Y. Arakawa, and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1967.
[750] J. B. Beadle, *J. Chromatog.*, 1969, **42**, 201.

has been shown to be ultrasensitive to carbonyl-containing carbohydrate derivatives. The trimethylsilyl ether of *myo*-inosose-2 showed a response five hundered times that of non-carbonyl analogues, and the detection was therefore applied in a technique for assaying such compounds in biological tissues. A product, believed to be the acyclic penta-ether, was detected in trimethylsilylated D-fructose.[751]

The use of DMF or DMSO as solvents for trimethylsilylation in two-phase reactions has already been referred to;[175] another development described the trimethylsilylation of polyhydric alcohols in the presence of water. The hydrogenolysis products of sucrose were examined by use of the method.[752] Other workers have used a 5 : 5 : 1 mixture of *N*,*O*-bis(trimethylsilyl)acetamide, *N*-trimethylsilylimidazole, and trimethylsilyl chloride as a trimethylsilylating agent for examining the products of the Maillard browning reactions.[753]

The resolutions between the TMS ethers of many monosaccharides were found to be best with XE-60 as stationary phase;[754] other workers have recommended tetracyanoethyl pentaerythritol.[755]

The combined application of g.l.c. and mass spectrometry continues to be used, and has been applied to the analysis of TMS ethers of the methyl glycosides and alditols derived from 2-amino-2-deoxy-D-glucose and -D-galactose,[756] and also to partially methylated methyl glycosides, with which it was found that the positions of the methyl groups largely determined the nature of the spectra obtained.[757] Reduction and trimethylsilylation of disaccharides followed by g.l.c. and mass spectrometry are reported to be a convenient method for structural analysis. By these procedures the nature of the intermolecular bond, the molecular weight, and several other features can be established. Mass spectrometric analysis was facilitated by use of borodeuteride as reducing agent.[758]

G.l.c. of trimethylsilyl ethers has also been used in the analysis of the following: methyl ester methyl glycosides of hexuronic acids,[759] methyl ethers of D-glucose,[760] 2-amino-2-deoxyhexoses (initially deaminated with nitrous acid),[761] free sugars in plants (together with amino- and other organic acids),[762] glucose and fructose in honey [763] and in plant extracts,[764]

[751] W. R. Sherman and S. L. Goodwin, *J. Chromatog.*, 1969, **7**, 167.
[752] G. van Ling, *J. Chromatog.*, 1969, **44**, 175.
[753] M. L. Wolfrom and N. Kashimura, *Carbohydrate Res.*, 1969, **11**, 151.
[754] W. C. Ellis, *J. Chromatog.*, 1969, **41**, 335.
[755] D. Farshtchi and C. W. Moss, *J. Chromatog.*, 1969, **42**, 108.
[756] J. Kärkkäinen and R. Vihko, *Carbohydrate Res.*, 1969, **10**, 113.
[757] K. Heyns, K. R. Sperling, and H. F. Grützmacher, *Carbohydrate Res.*, 1969, **9**, 79.
[758] J. Kärkkäinen, *Carbohydrate Res.*, 1969, **11**, 247.
[759] J. R. Clamp and J. E. Scott, *Chem. and Ind.*, 1969, 652.
[760] S. Haworth, J. G. Roberts, and B. F. Sagar, *Carbohydrate Res.*, 1969, **9**, 491.
[761] S. Hase and Y. Matsushima, *J. Biochem. (Japan)*, 1969, **66**, 57.
[762] K. Salminen and P. Koivistoinen, *Acta Chem. Scand.*, 1969, **23**, 999.
[763] Y. Masada, K. Hashimoto, T. Inoue, and T. Sawada, *J. Pharm. Soc. Japan*, 1969, **89**, 734.
[764] P. K. Davison and R. Young, *J. Chromatog.*, 1969, **41**, 12.

mono- and di-saccharides and alditols in foodstuffs and biological fluids,[765] mono-, di-, and tri-saccharides in potato tubers,[766] lactulose in urine,[767] acid products formed by oxidation of hexoses in alkaline solution,[768] and neuraminyl oligosaccharides.[769]

The separation of the TMS ethers of L-ascorbic acid and D-*arabino*-ascorbic acid has been described,[770] as have those of the sixteen heptono-1,4-lactones.[771]

Column and Ion-exchange Chromatography.—A method employing Sephadex gel filtration and paper chromatography has been developed for the separation and determination of the oligosaccharides of human milk. The four oligosaccharides which contain *O*-α-L-fucosyl-(1 → 2)-*O*-β-D-galactosyl groupings occurred in milk from donors with blood type Le(b$^+$) but not in milk from Le(a$^+$) donors.[772] Sephadex separations have also been carried out on phenolic glycosides,[773] inositol phosphates,[578] and monosaccharides and oligosaccharides. Glucose homologues up to D.P. 13 were separable within a few hours.[774]

A review has appeared on the ion-exchange chromatography of sugars and their derivatives.[775] The method has been applied to the separation of mono-, di-, and tri-saccharides (columns eluted with borate–butane-2,3-diol),[776] 2-deoxy-D-*erythro*-pentose 5-phosphate, the D-ribose ester,[777] and 2-amino-2-deoxy-D-glucose and -galactose. The last two sugars and their corresponding alditols were examined with an automatic amino-acid analyser, and a method for their determination in reduced hydrolysates of mucopolysaccharides was developed.[778] An automated technique for determining erythritol and threitol involved ion-exchange separations.[779]

Paper Chromatography and Electrophoresis.—A convenient and sensitive method for detecting non-reducing carbohydrates on paper chromatograms with silver nitrate–potassium hydroxide after periodate oxidation has been described,[780] and a technique was developed for the quantitative analysis of glucose, galactose, and their 2-amino-2-deoxy analogues, and

[765] J. De Neef, *Clinica Chim. Acta*, 1969, **26**, 485.
[766] M. Kimura, M. Tohma, Y. Okazawa, and N. Murai, *J. Chromatog.*, 1969, **41**, 110.
[767] M. Müller, J. Walker-Smith, D. H. Shmerling, H.-Ch. Curtius, and A. Prader, *Clinica Chim. Acta*, 1969, **24**, 45.
[768] L. A. Th. Verhaar and H. G. J. de Wilt, *J. Chromatog.*, 1969, **41**, 168.
[769] J. K. Huttunen and T. A. Miettinen, *Analyt. Biochem.*, 1969, **29**, 441.
[770] T. Anmo, M. Washitake, H. Hayashi, H. Yamaguchi, and A. Miyano, *J. Pharm. Soc. Japan*, 1969, **89**, 1308.
[771] M. B. Perry, G. A. Adams, and D. H. Shaw, *J. Chromatog.*, 1969, **44**, 614.
[772] A. Kobata and V. Ginsburg, *Arch. Biochem. Biophys.*, 1969, **130**, 509.
[773] A. Repaš, B. Nikolin, and K. Dursun, *J. Chromatog.*, 1969, **44**, 184.
[774] M. John, G. Trénel, and H. Dellweg, *J. Chromatog.*, 1969, **42**, 476.
[775] O. Samuelson, *Ion Exchange*, 1969, **2**, 167.
[776] E. F. Walborg jun., D. B. Ray, and L. E. Öhrberg, *Analyt. Biochem.*, 1969, **29**, 433.
[777] N. L. Fortier, J. Silva, and F. J. Lionetti, *Analyt. Biochem.*, 1968, **26**, 490.
[778] P. Weber and R. J. Winzler, *Arch. Biochem. Biophys.*, 1969, **129**, 534.
[779] S. A. Barker, M. J. How, P. V. Peplow, and P. J. Somers, *Analyt. Biochem.*, 1968, **26**, 219.
[780] A. I. Usov and M. A. Rekhter, *Zhur. obshchei. Khim.*, 1969, **39**, 912.

of mannose and glucuronic acid, which are found in the hydrolysates of fungal cell walls. Glass-fibre paper impregnated with silica gel and sodium dihydrogen phosphate was used as the chromatographic support, and the sugars were detected with sulphuric acid and determined by densitometry.[781]

The streaking of 2-amino-2-deoxy-D-mannose hydrochloride on paper chromatograms has been examined by use of radioactive sugar, and it was concluded that the problem is inherent to the compound.[782]

Methods for the separation of L-ascorbic acid phosphates by paper electrophoresis have been described.[783]

Thin-layer Chromatography.—A spot test specific for hydroxy-groups has been developed, based on 4-(*p*-nitrobenzyl)pyridine sprayed on to cellulose t.l.c. plates.[784]

The separation of twenty-five sugars on silica, impregnated with sodium acetate, monosodium phosphate, and disodium phosphate, has been investigated. Excellent separations of up to ten sugars were reported, and conditions for the resolution of biological extracts were developed.[785] A method for the separation of sugars in the presence of large proportions of sucrose has been described,[786] separations of free sugars by 1- and 2-dimensional t.l.c. on cellulose and starch have been investigated,[787] and a system for separating fucose and *N*-acetylhexosamines has been reported.[788] Double-development procedures and two-dimensional techniques for resolving mixtures of di-, tri-, and tetra-saccharides have been studied,[789] as have methods of separating such compounds obtained from barley grain.[790]

The mobilities of one hundred and sixty cardiac glycosides and genins and their peracetates have been examined in different solvent systems, and the possibility of developing a structural analytical method based on t.l.c. characteristics was discussed.[791]

Further reports have described the separations of carbohydrate-containing antibiotics by t.l.c. on Sephadex,[792] and the resolutions of *ribo*-, 2′-deoxy-D-*erythro*-pento-, and *arabino*-nucleosides.[793]

[781] S. J. Kraeger and J. G. Hamilton, *J. Chromatog.*, 1969, **41**, 113.
[782] G. Sheppard, *J. Chromatog.*, 1969, **40**, 312.
[783] M. Shimomura, I. Aoki, M. Miyazaki, M. Yasumatsu, M. Hori, and M. Hattori, *Ann. Rep. Takeda. Res. Zeb.*, 1968, **27**, 54 (*Chem. Abs.*, 1969, **70**, 78,292*m*).
[784] J. G. Pomonis, R. F. Severson, and P. J. Freeman, *J. Chromatog.*, 1969, **40**, 78.
[785] M. Lato, B. Brunelli, G. Ciuffini, and T. Mezzetti, *J. Chromatog.*, 1969, **39**, 407.
[786] P. D. Berger and A. S. Agate, *J. Chromatog.*, 1969, **39**, 232.
[787] S. M. Petrovíc and V. D. Caníc, *Mikrochim. Acta*, 1969, 599.
[788] K. Hotta and M. Kurokawa, *Analyt. Biochem.*, 1968, **26**, 472.
[789] D. C. Jeffrey, J. Arditti, and R. Ernst, *J. Chromatog.*, 1969, **41**, 475.
[790] J. Washüttl, *Mikrochim. Acta*, 1969, 1003.
[791] L. Nover, G. Jüttner, S. Noack, G. Baumgarten, and M. Luckner, *J. Chromatog.*, 1969, **39**, 419, 450.
[792] M. H. J. Zuidweg, J. G. Oostendorp, and C. J. K. Bos, *J. Chromatog.*, 1969, **42**, 552.
[793] D. W. Jacobsen and J. Kirchner, *Analyt. Biochem.*, 1968, **26**, 474.

Other Analytical Methods

Several methods based on well-known procedures have been reported for the determination of aldoses by enzymic methods. D-Glucose oxidase has been used to determine D-glucose,[794] and in several procedures specific features were included. Thus, one technique used polarography to determine the oxygen consumed during the reaction,[795] one used a titrimetric method, one considered the effect of sulphydryl reagents,[796] and another considered the fragmentation of the ^{14}C-labelled sugar.[797] Enzymic methods have also been developed for the assay of D-arabinose, D-galactose, and their homologues.[798]

Several colorimetric methods have been explored or adapted; methods based on silicomolybdic acid,[799] *NN*-diethyl-*p*-phenylenediamine,[800] cysteine–sulphuric acid [801] have been used as general methods for determining free sugars, and automated processes were also described.[802, 803] Glucose [804] and xylose [805] have been assayed using *O*-toluidine, and a method for determining glucose, fructose, and sucrose based on the different rates at which they produced hydroxymethylfurfural with acid has been developed.[806] Four sulphuric acid-based charring techniques have been evaluated for their suitability in quantitative analysis of carbohydrates by transmission densitometry.[807]

Volumetric methods have been used for the assay of blood glucose in the presence of acetoacetate (ferricyanide used),[808] and gold chloride has been employed in a titrimetric microanalytical method for pentoses.[809]

A method based on the precipitation of the polymer was developed for the determination of glucose and glycogen in meat.[810]

Ketoses have received specific attention. One procedure assayed ketohexoses titrimetrically with alkaline gold chloride,[811] one used anthrone for the assay of fructose in biological fluids,[811a] one determined fructose

[794] S. E. Aw, *Clinica Chim. Acta*, 1969, **26**, 235.
[795] J. Okuda and G. Okuda, *Clinica Chim. Acta*, 1969, **23**, 365.
[796] D. M. Kilburn and P. M. Taylor, *Analyt. Biochem*, 1969, **27**, 555.
[797] R. Rognstad and J. Woronsberg, *Analyt. Biochem.*, 1968, **25**, 448.
[798] A. S. L. Hu and S. Grant, *Analyt. Biochem.*, 1968, **25**, 221.
[799] V. N. Khrustaleva, M. F. Kachalova, and V. V. Kozlov, *Izvest. V. U. Z., Pishch. Tekhnol*, 1968, 38 (*Chem. Abs.*, 1968, **69**, 106,985*q*).
[800] R. H. Thompson, *Clinica Chim. Acta*, 1969, **15**, 475.
[801] R. J. Thibert and A. Mazzuchin, *Canad. J. Biochem.*, 1969, **47**, 203.
[802] A. Tsuti, T. Kinoshita, A. Sakai, and M. Hoshino, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1305.
[803] J. W. Hall and D. M. Tucker, *Analyt. Biochem.*, 1968, **26**, 12.
[804] G. Ceriotti and A. de N. Frank, *Clinica Chim. Acta*, 1969, **24**, 311.
[805] A. L. Harris, *Clinical Chem.*, 1969, **15**, 65.
[806] E. R. Garrett and J. F. Young, *J. Pharm. Sci.*, 1969, **58**, 1224.
[807] J. Lehrfeld and J. C. Goodwin, *J. Chromatog.*, 1969, **45**, 150.
[808] J. G. Salway, *Clinica Chim. Acta*, 1969, **25**, 109.
[809] U. Shanker, R. K. Srivastava, and O. C. Saxena, *J. Chem. U.A.R.*, 1969, **12**, 145.
[810] P. M. Hefferan and K. C. Goodnight, *J. Food Sci.*, 1969, **34**, 353.
[811] O. C. Saxena, *Microchem. J.*, 1968, **13**, 571.
[811a] D. A. Nixon, *Clinica Chim. Acta*, 1969, **26**, 167.

by an automated process in the presence of glucose,[812] one likewise determined D-mannoheptulose automatically,[813] and another assayed 3-keto-sucrose and D-*ribo*-hexos-3-ulose in conditions under which D-fructose did not interfere.[814]

A highly sensitive method for determining hexosamines is based on their reaction with 3-methyl-2-benzothiazolone hydrazone hydrochloride and ferric chloride,[815, 816] and another used *p*-nitrobenzaldehyde and tetraethylammonium hydroxide.[817] It has been demonstrated that amino-acid products can interfere with the Elson–Morgan test for hexosamines.[818]

An analytical process for determining mannitol and glucitol has been based on polarimetric examination of their molybdate complexes.[819]

Uronic acids can be detected on the automatic amino-acid analyser,[820] and several quantitative tests for ascorbic acid have been established. These depend upon colorimetric reactions with 2,6-dichlorophenol–indophenol,[821] $\alpha\alpha'$-dipyridyl, and ferric chloride,[822] and diazotised 4-methoxy-2-nitroaniline.[823] A further spectrophotometric method has been established,[558] and the bromate–iodide titrimetric procedure has been developed.[824]

Critical comments have been published on the assay of D-galactose 1-phosphate,[825] and the utility of flame photometry for the determination of barium in carbohydrate sulphate salts has been assessed.[826]

The quantitative analysis of nucleosides and nucleotides by reflectance spectroscopy after t.l.c. separation has been reported.[827]

The colour generated with benzhydrazide has been used in an assay for the periodate ion,[828] and a similar approach was based on the oxidation of the violet ferrous 2,4,6-tripyridyl-*s*-triazine to a colourless product.[829]

An automated method for the direct determination of periodate-resistant carbohydrate residues used sodium bisulphite to destroy excess of the

[812] M. C. Cadmus and G. W. Strandberg, *Analyt. Biochem.*, 1968, **26**, 487.
[813] J. Viktora, K. Weinhardt, and F. Wolff, *Analyt. Biochem.*, 1968, **26**, 299.
[814] S. Fukui and K. Hayano, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 1013.
[815] A. Tsuji, T. Kinoshita, and M. Hoshino, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 217.
[816] A. Tsuji, T. Kinoshita, and M. Hoshino, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1505.
[817] A. Nakamura, M. Maeda, T. Kinoshita, and A. Tsuji, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 770.
[818] G. Chotiner, J. G. Smith jun., and E. A. Davidson, *Analyt. Biochem.*, 1968, **26**, 146.
[819] M. Hamon, C. Morin, and R. Bourdon, *Analyt. Chim. Acta*, 1969, **46**, 255.
[820] A. C. Olson, L. M. White, and A. T. Noma, *J. Chromatog.*, 1969, **43**, 399.
[821] H. Vallant, *Mikrochim. Acta*, 1969, 436.
[822] S. Baczyk and L. Duczmal, *Mikrochim. Acta*, 1968, 1291.
[823] G. Geller, O. W. A. Weber, and B. Z. Senkowski, *J. Pharm. Sci.*, 1969, **58**, 477.
[824] J. Bognár and O. Jellinek, *Mikrochim. Acta*, 1969, 312.
[825] V. Schwarz and I. M. N. Simpson, *Clinica Chim. Acta*, 1969, **24**, 188.
[826] P. F. Lloyd, B. Evans, and R. J. Fielder, *Carbohydrate Res.*, 1969, **11**, 129.
[827] R. W. Frei, H. Zürcher, and G. Pataki, *J. Chromatog.*, 1969, **45**, 284.
[828] A. M. Escarrilla, P. F. Maloney, and P. M. Maloney, *Analyt. Chim. Acta*, 1969, **45**, 199.
[829] G. Avigad, *Carbohydrate Res.*, 1969, **11**, 119.

reagent, and a modified cysteine–sulphuric acid method for estimating intact sugars. The formaldehyde produced was also monitored;[830] another formaldehyde analytical procedure has also been reported.[831]

It has been found that the periodate–thiobarbituric acid reaction is not specific for neuraminic acids, and that all 3-deoxyulosonic acids give a positive reaction.[536]

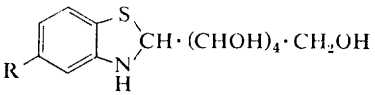
[830] P. V. Peplow and P. J. Somers, *Carbohydrate Res.*, 1969, **9**, 33.
[831] V. E. Vaskovsky and S. V. Isay, *Analyt. Biochem.*, 1969, **30**, 25.

27
Alditols

The crystal structures of ribitol,[732] and of xylitol,[733] have already been described. The c.d. spectra of molybdate complexes of polyols have been studied[740] at various pH values; characteristic behaviour was shown by compounds with the *arabino* configuration at C-3, C-4, and C-5. The aqueous boric acid–borate–mannitol system has been reinvestigated, and the results of previous workers reinterpreted. It was concluded that both 1 : 1 and 1 : 2 mannitol : borate complexes were involved.[832]

Treatment of 3,4-anhydro-D-allitol with dilute aqueous sodium hydroxide gave 1,4-anhydro-D-allitol and 1,5-anhydro-L-glucitol as the sole products; 2,3-anhydro-D-iditol was postulated as an intermediate.[833] Other papers on anhydro-alditols have already been mentioned,[180a, 715, 727] as well as papers on acetals[200, 716] and esters.[230, 249]

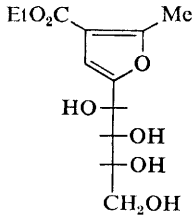
Acetylation of riboflavin with acetone gave[834] the following new acetals: 2,4:3,5 ; 4,5 ; 3,4 ; and 2,3. 2-(Polyhydroxyalkyl)-benzothiazolines, for example (492), with the *gluco* or *galacto* configuration have been prepared



(492) R = H or Cl

by the condensation of aldoses, or aldose *aldehydo*-peracetates, with 2-amino-thiophenol or with 2-amino-4-chloro-thiophenol.[835] The same group have also studied the synthesis of 2-(polyhydroxyalkyl)-thiazoles.[836] Acetylation of compound (493) has been investigated.[837]

[832] J. Knoeck and J. K. Taylor, *Analyt. Chem.*, 1969, **41**, 1730.
[833] J. G. Buchanan and A. R. Edgar, *Carbohydrate Res.*, 1969, **10**, 295.
[834] T. Tanaka, H. Tanaka, and T. Tsubaki, *J. Pharm. Soc. Japan*, 1968, **88**, 1313.
[835] R. Bognár, Z. Kolodynska, L. Somogyi, Z. Györgyadeák, L. Szilágyi, and É. N. Nemes, *Acta Chim. Acad. Sci. Hung.*, 1969, **62**, 65.
[836] R. Bognár, I. Farkas, L. Szilágyi, M. Menyhart, É. N. Nemes, and I. F. Szabó, *Acta Chim. Acad. Sci. Hung.*, 1969, **62**, 179.
[837] A. Gómez Sánchez, M. López Artiguez, A. Rodríguez Roldán, and F. García Gonzalez, *Anales de Quím.*, 1968, **64**, 1077.



(493)

Application of the Curtius degradation to octa-*O*-acetyl-cellobionic acid azide gave 1-acetamido-2,3,5,6-tetra-*O*-acetyl-1-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucitol, which was degraded further to 1,1-bis(acetamido)-1-deoxy-3-*O*-(β-D-glucopyranosyl)-D-arabinitol.[838]

Alditol compounds continue to be widely used as derivatives for g.l.c. analysis; see Chapter 26.

[838] I. Farkas, I. F. Szabó, and R. Bognár, *Acta Chim. Acad. Sci. Hung.*, 1969, **59**, 41.

Part II

MACROMOLECULES

By

P. J. Somers

1
Introduction

The objectives stated in previous volumes have been pursued in the preparation of this volume. The same general arrangement of topics has been adopted as in previous volumes of this series. In view of the increasing interest in hydrolytic enzymes, particularly with respect to structural investigations on polysaccharides, a new chapter has been introduced to cover those enzymes whose isolation and action patterns are of interest.

2
General Methods

Analysis*

A number of methods have been reported for the determination of carbohydrates in macromolecules by g.l.c., after conversion to suitable derivatives. Direct trimethylsilylation with bis-(trimethylsilyl)trifluoroacetamide permitted the determination of 3-deoxy-D-*manno*-octulosonic acid in several lipopolysaccharide preparations from Gram-negative bacteria.[1] Naturally-occurring hexuronic acids were determined by g.l.c. of the corresponding methyl ester methyl glycosides, after formation of trimethylsilyl derivatives.[2] 2-Amino-2-deoxy-hexoses could be simultaneously analysed after conversion to the corresponding trimethylsilylated 2-acetamido-2-deoxy-hexose. 4-*O*-Methyl-D-glucuronic acid was determined in a wood xylan after esterification with propylene oxide, borohydride reduction, and sulphuric acid hydrolysis.[3] Conversion to the monomethylated alditol acetate, and separation by g.l.c., provided a suitable derivative for mass spectral analysis. The enzymic degradation products of commercial pectins, particularly D-galacturonic acid, were analysed as their trimethylsilyl derivatives.[4] A combination of g.l.c. and mass spectroscopy has been employed for the determination of the trimethylsilyl derivatives of 2-acetamido-2-deoxy-D-glucose and -D-galactose in polysaccharides.[5]

A study has been made of the acid stability of monosaccharides with reference to the analysis of glucose, galactose, and mannose in polysaccharides.[6] The recovery of these hexoses from the methanolysis of a mannan, a glucomannan, and a galactomannan–peptide complex was assessed by g.l.c. analysis of the trimethylsilylated methyl glycosides. It was concluded that no satisfactory conditions were available for complete recovery of the hexoses from the methanolysis of polysaccharides. α-, β-, and γ-Cyclodextrins were determined by g.l.c. of the corresponding dimethylsilyl ethers.[7] It was concluded that the formation of multiple

[1] D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 691.
[2] J. R. Clamp and J. E. Scott, *Chem. and Ind.*, 1969, 652.
[3] E. Sjostrom, S. Juslin, and E. Seppala, *Acta Chem. Scand.*, 1969, **23**, 3610.
[4] R. C. Wiley and M. Tavboli, *Food Technol.*, 1969, **23**, 167.
[5] J. Karkkainen and R. Vihko, *Carbohydrate Res.*, 1969, **10**, 113.
[6] Y. Nozawa, Y. Hiraguri, and Y. Ito, *J. Chromatog.*, 1969, **45**, 244.
[7] J. B. Beadle, *J. Chromatog.*, 1969, **42**, 201.

* See also Part I, Chapter 26.

peaks after trimethylsilylation was due to steric hindrance to etherification. Methanolysis, followed by trimethylsilylation and g.l.c. analysis, has been used to determine the carbohydrate constituents of bacterial endotoxins.[8] A method has been described for the analysis of urinary neuraminyl oligosaccharides by a combination of ion-exchange chromatography and g.l.c. of the trimethylsilyl ethers.[9]

A fluorimetric–enzymic method has been reported for the analysis of inulin and D-glucose.[10] D-Galactose and certain D-galactose derivatives were determined by reaction with D-galactose dehydrogenase from *Pseudomonas saccharophila*.[11] 2-Amino-2-deoxy-hexoses have been determined by conversion to 2,5-anhydro-hexoses with nitrous acid and subsequent reaction with 3-methyl-2-benzothiazolone hydrazone.[12] An improved procedure has been devised for the determination of carbohydrates in glycoprotein hydrolysates by reaction with 2,3,5-triphenyltetrazolium chloride on chromatograms.[13]

Further methods have been reported for the automated spectrophotometric determination of hexoses and hexose derivatives. Two alternative procedures have been described for the automated analysis of hexuronic acids in glycosaminoglycans using the carbazole–sulphuric acid reaction.[14] The influence of serum components on the determination of hexuronic acids by the borate modification of the carbazole–sulphuric acid reaction has been determined.[15] A limitation in heating time was desirable to minimise interference. A combination of ion-exchange chromatography with an automated determination by a neocuproine reducing sugar method was used for the determination of 2-amino-2-deoxy-hexoses in glycoprotein hydrolysates.[16] A similar method has been established for the determination of neutral sugars in glycoprotein hydrolysates.[17] An improved method for the determination of mono-, di-, and tri-saccharides has been described.[18] Elution of the borate complexes from an ion-exchange resin with boric acid–butan-2,3-diol buffers allowed separation of a range of compounds.

Cellulose was determined in biological materials by the anthrone method after extraction of lignin, hemicellulose, and xylans with an acetic acid–nitric

[8] C. E. Davis, S. D. Freedman, H. Douglas, and A. Braude, *Analyt. Biochem.*, 1969, **28**, 243.
[9] J. K. Hattunen and T. A. Miettinen, *Analyt. Biochem.*, 1969, **29**, 441.
[10] R. Zwiebel, B. Hohmann, P. Frohnert, and K. Baumann, *European J. Physiol.*, 1969, **307**, 127.
[11] G. Kurz, *Z. Analyt. Chem.*, 1969, **243**, 258.
[12] A. Tsuji, T. Kinashita, and M. Hoshino, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 217.
[13] J. Mes and L. Kamm, *J. Chromatog.*, 1969, **43**, 480.
[14] K. von Berlepsch, *Analyt. Biochem.*, 1969, **27**, 424.
[15] G. Harris and J. R. E. Fraser, *Analyt. Biochem.*, 1969, **27**, 433.
[16] Y. C. Lee, J. R. Scocca, and L. Muir, *Analyt. Biochem.*, 1969, **27**, 559.
[17] Y. C. Lee, J. F. McKelvy, and D. Lang, *Analyt. Biochem.*, 1969, **27**, 567.
[18] E. F. Walborg jun., D. B. Ray, and L. E. Ohrberg, *Analyt. Biochem.*, 1969, **29**, 433.

acid mixture.[19] A spectrophotometric method has been described for the determination of trityl groups in polymeric carbohydrates.[20] A flame photometric method for the determination of sulphate in carbohydrate sulphates has been described.[21]

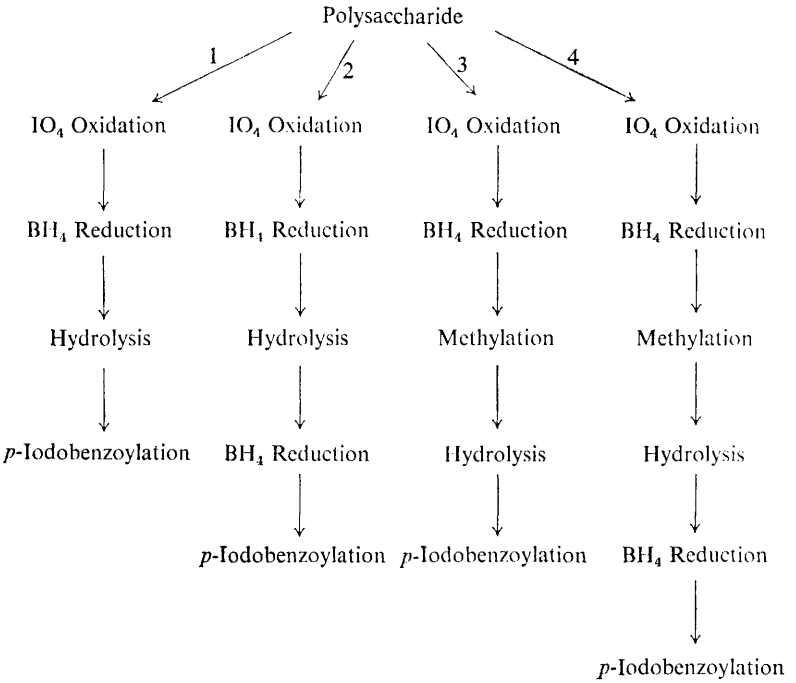
T.l.c. systems have been described for the separation of the methanolysis products of polysaccharide–peptide complexes,[22] maltodextrins,[23] and bacterial cell-wall hydrolysates.[24] Glass–paper chromatography has been recommended for the separation of the products of fungal cell-wall hydrolysis.[25]

α- and β-Cyclodextrins have been reproducibly separated on columns of charcoal, the separation and reproducibility being improved by flotation of the charcoal to remove fine particles.[26] Automated quantitative chromatography of glucose oligomers on polyacrylamide gel (P2) has been described. Maltodextrins of DP up to 13 were separated from amylomaltase action on maltose.[27] The automated molecular sieve chromatography of polysaccharides has been described.[28] The separation of carbohydrates on cation exchange resins, based on ion exclusion and gel permeation, has been described.[29] Inorganic ions and acidic carbohydrates were excluded from the resin matrix. The fractionation of dextran and hyaluronic acid on porous silica beads has been evaluated. The effect of sample concentration, flow rate, and temperature were examined and the relation between molecular weight and elution position established.[30] A system has been described for the automated analysis of total and end-group concentration for the fractionation of dextran on porous silica.[31] The values of M_n obtained when plotted against elution position showed significant deviations from the S-shaped curves normally used for calibration.

Structural Methods

A scheme for the determination of bond-types in polysaccharides has been discussed.[32] Fragments obtained after periodate oxidation and borohydride reduction were converted into alditol *p*-iodobenzoates to allow identification (Scheme 1). It was suggested that use of [^{131}I] would facilitate reverse

[19] D. M. Updegraff, *Analyt. Biochem.*, 1969, **32**, 420.
[20] H. C. Srivastava, K. V. Ramalingam, and S. Chakrabarti, *Indian J. Chem.*, 1969, **7**, 98.
[21] P. F. Lloyd, B. Evans, and R. J. Fielder, *Carbohydrate Res.*, 1969, **11**, 129.
[22] Y. Nozawa, H. Uesaka, H. Suzuki, and Y. Ito, *J. Chromatog.*, 1969, **43**, 528.
[23] C. E. Weill, *Cereal Chem.*, 1969, **46**, 177.
[24] A. S. Bleiweiss and S. E. Coleman, *Analyt. Chem.*, 1969, **29**, 347.
[25] S. J. Kraeger and J. G. Hamilton, *J. Chromatog.*, 1969, **41**, 113.
[26] J. N. J. J. Lammers, *J. Chromatog.*, 1969, **41**, 462.
[27] M. John, G. Trenel, and H. Dellweg, *J. Chromatog.*, 1969, **42**, 476.
[28] D. M. W. Anderson, A. Hendrie, and A. C. Munro, *J. Chromatog.*, 1969, **44**, 178.
[29] S. A. Barker, B. W. Hatt, J. F. Kennedy, and P. J. Somers, *Carbohydrate Res.*, 1969, **9**, 327.
[30] S. A. Barker, B. W. Hatt, J. B. Marsters, and P. J. Somers, *Carbohydrate Res.*, 1969, **9**, 373.
[31] S. A. Barker, B. W. Hatt, and P. J. Somers, *Carbohydrate Res.*, 1969, **11**, 355.
[32] P. Nanasi and A. Liptak, *Carbohydrate Res.*, 1969, **10**, 177.



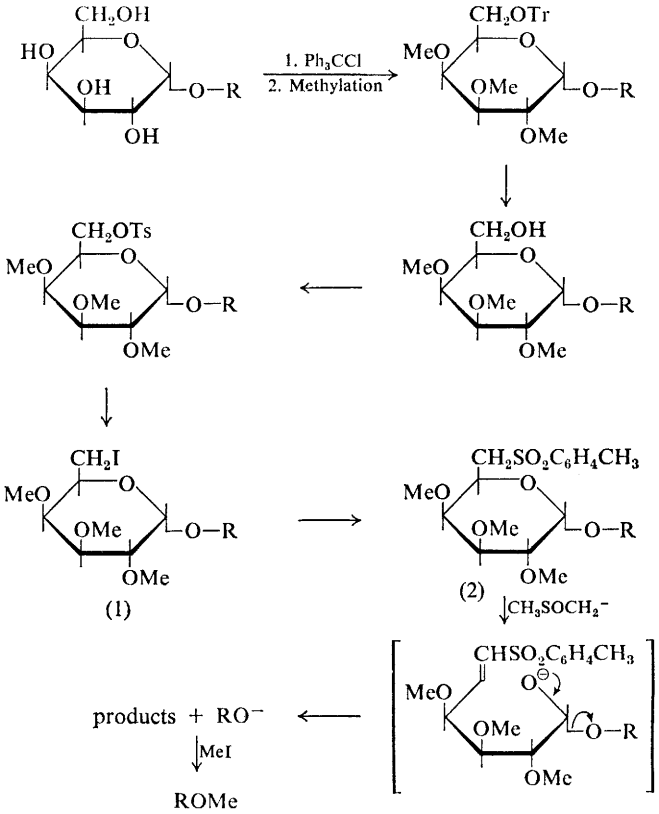
Scheme 1

isotope dilution and allow identification and quantitative determination of the derivatives.

Two techniques have been investigated for the selective degradation of polysaccharides. Model experiments with dextran were designed to investigate glycoside stabilisation with toluene-*p*-sulphonates.[33] Substitution of the primary hydroxy-groups of polysaccharides was proposed to stabilise linkages to acid hydrolysis. Successive tritylation, methylation, detritylation, sulphonylation, and methanolysis of dextran gave mainly derivatives of isomaltose. *O*-Methyl-*O*-toluene-*p*-sulphonyl-oligosaccharides were difficult to characterise. Alkaline elimination of 6-*C*-(toluene-*p*-sulphonyl) residues in polysaccharides was used to achieve selective scission of the chain at residues having unsubstituted primary hydroxy-groups in the original polymer.[34] Successive tritylation, methylation, detritylation, sulphonylation, and a displacement reaction gave the 6-deoxy-6-iodo-compound (1) (Scheme 2). Reaction of (1) with toluene-*p*-sulphinyl chloride gave the 6-deoxy-6-toluene-*p*-sulphonyl derivative (2).

[33] D. A. Rees, N. G. Richardson, N. J. Wight, and Sir Edmund Hirst, *Carbohydrate Res.*, 1969, **9**, 451.

[34] H. Bjorndal and B. Wagstrom, *Acta Chem. Scand.*, 1969, **23**, 3313.



Scheme 2

Reaction with methylsulphinyl sodium in DMSO resulted in elimination of the substituted residues and released alkoxide ions, subsequently methylated with methyl iodide. The method was employed to investigate a hetero glycan from *Polyporus squamosus*.

An apparatus has been described for the continuous collection of oligosaccharides produced on partial acid hydrolysis of polysaccharides to improve the yield of acid-labile oligosaccharides.[35] Continuous dialysis removed the oligomers which were then absorbed on a column of charcoal. Monosaccharides were not absorbed on the column, thus effecting a concentration of the oligosaccharides.

Hemicelluloses of *Cynodon plectostachyus* and *Setaria sphacelata* have been methylated, as model compounds, by various procedures to investigate their suitability for structural investigations.[36] Reaction of the polysaccharides with sodium hydride–DMSO–methyl iodide gave a high yield

[35] C. Gakanos, O. Luderitz, and K. Himmelspach, *European J. Biochem.*, 1969, **8**, 332.
[36] N. W. H. Cheetham and R. J. McIlroy, *Carbohydrate Res.*, 1969, **11**, 187.

(90—100%) of methylated polymers. Use of the Haworth procedure, followed by the Purdie procedure, required multiple reactions to achieve complete methylation with low overall yields (39—42%). It was also found that reaction with sodium hydroxide–DMSO–dimethyl sulphate was unsatisfactory since considerable depolymerisation occurred, and extraneous compounds were found on methanolysis of the product.

Manual and automated techniques for the direct spectrophotometric determination of periodate-resistant sugar units in periodate oxidation mixtures have been devised.[37] The methods were used to monitor the periodate oxidation of lactitol, maltitol, and nigeran. The use of the periodate–Schiff spray reagent to enable rapid linkage analysis of oligosaccharides has been described.[38] The method was suitable for any hexopyranose disaccharide and certain higher homologues.

A quantitative correlation has been demonstrated between the chemical composition and observed optical rotation of a series of water-soluble galactomannans.[39] A quantitative relation was calculated between the optical rotation and the molar ratio of mannose to galactose for galactomannans having a β-(1 → 4)-D-mannopyranosyl backbone with α-(1 → 6)-D-galactopyranosyl side-chains based on the principle of optical superposition. One group of galactomannans gave a good correlation, but a second group did not, due to presumed structural anomalies. Study of the o.r.d. and c.d. curves of mono-, oligo-, and poly-saccharides containing amino-sugars and their derivatives has allowed tentative rules to be postulated for the determination of the nature and position of substituents on 2-acetamido-2-deoxy-D-glucose units in oligo- and poly-saccharides.[40] 2-Acetamido-2-deoxy-D-glucose units in teichoic acids dominated the shape and largely determined the intensity of the o.r.d. curves. Although o.r.d. and c.d. did not distinguish between mixtures of α- and β-teichoic acids and molecules with both linkages on a common ribitol phosphate backbone, determination of the proportion of α- and β-linked units was possible.

Correlations have been made between the n.m.r. spectra and chemical structures of a variety of cell-wall mannans and galactomannans of yeasts.[41]

Specific Interactions of Carbohydrates with Concanavalin A

Further studies have been reported on the nature of the reaction of concanavalin A with carbohydrates and its use in structural investigations. Study of the effect of polysaccharide molecular weight on the precipitation reaction indicated that equivalence was reached earlier with lower molecular

[37] P. V. Peplow and P. J. Somers, *Carbohydrate Res.*, 1969, **9**, 33.
[38] A. R. Archibald and J. G. Buchanan, *Carbohydrate Res.*, 1969, **11**, 558.
[39] C. Leschziner and A. S. Cerezo, *Carbohydrate Res.*, 1969, **11**, 113.
[40] E. A. Kabat, K. O. Lloyd, and S. Beychok, *Biochemistry*, 1969, **8**, 747.
[41] P. A. J. Gorin, J. F. T. Spencer, and R. J. Magus, *Canad. J. Chem.*, 1969, **47**, 3569.

weight polysaccharides.[42] Furthermore, lower molecular weight polysaccharides were more effective in inhibition of precipitation in the region of polysaccharide excess. Interaction of concanavalin A with D-fructans (levans) has been demonstrated.[43] These polymers were less reactive than dextrans, mannans, glycogens, and amylopectins. The terminal β-D-fructofuranosyl moiety of the levans contains a common feature (at $C_3—C_4—C_6$ or $C_1—C_3—C_4$) to the $C_3—C_4—C_6$ portion of α-D-glucopyranosyl end-groups in α-D-glucans, and accounted for the reaction. On the basis of the extensive reactions now known, the generalisation was made that all molecules containing the 1,4-anhydro-D-arabinitol moiety will bind with concanavalin A.[43]

The reaction of concanavalin A with IgM and glycoprotein phytohemagglutinins of wax bean and soy bean suggested that the reactive carbohydrate residues were on the surface of the glycoproteins.[44] Serologically active, chemically distinct polysaccharides extracted from various strains of *Histoplasma capsulatum* were not distinguished in their reaction with concanavalin A.[45] It was not established if these galactomannans (containing traces of glucose) failed to react due to relative insensitivity of concanavalin A or the presence of only slight structural differences.

Interaction with concanavalin A has been used in the study of the immunochemistry of blood-group substances. The majority of hog stomach lining blood-group substances and some from human stomach linings precipitated, whereas human ovarian cyst material did not precipitate.[46] The isolation of the inhibitory disaccharide 3- or 4-*O*-(α-2-acetamido-2-deoxy-D-glucopyranosyl)-D-galactitol from hog gastric mucin by degradation with sodium hydroxide–sodium borohydride indicated the basis for this reaction. Two samples of hog gastric blood-group substance were fractionated by concanavalin A into two fractions, one of which had blood-group activity but did not precipitate and one of which was precipitated by anti-A and concanavalin A.

[42] L. L. So and I. J. Goldstein, *J. Immunol.*, 1969, **102**, 53.
[43] L. L. So and I. J. Goldstein, *Carbohydrate Res.*, 1969, **10**, 231.
[44] I. J. Goidstein, L. L. So, Y. Yang, and Q. C. Callius, *J. Immunol.*, 1969, **103**, 695.
[45] H. Markowitz, *J. Immunol.*, 1969, **103**, 308.
[46] K. O. Lloyd, E. A. Kabat, and S. Beychok, *J. Immunol.*, 1969, **102**, 1354.

3
Plant Polysaccharides

Further reports have appeared concerning the chemistry of the gum exudates of the genus *Araucaria*. An analytical study of the exudates from five species has been reported, and the data obtained compared with previously examined *Araucaria* species.[47] From a chemotaxonomic viewpoint no large differences were observed between the *Colymbea* and *Eutacta* sections of the genus. The observed differences were not as large as those in the genera of the Angiospermae. The comparatively rare 3-*O*-methyl-L-rhamnose was found in resinous extracts of five *Araucaria* species and appeared to act as a useful chemotaxonomic marker in the Gymnospermae.[48] The chemotaxonomic aspects of the chemistry of *Acacia* gum exudates have been reviewed and discussed with respect to Bentham's divisions of the genus.[49]

The application of g.l.c. to the structural investigation of polysaccharides has been studied using the polysaccharides from *Acacia podalyriafolia* A. Cunn. and *A. elata* A. Cunn.[50] M_w values for a series of *Acacia* gums have been obtained by light-scattering studies.[51] Some molecular aggregation has been observed in *Acacia* species gum exudates on freeze-drying.[52] This accounted for the high-molecular-weight fractions observed during molecular-sieve chromatography on Sepharose 4B.

Some structural features of brea gum (from *Cercidium australe* Jonhst.) have been established.[53] The polysaccharide contained L-arabinose, D-xylose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid (1·7 : 6·3 : 1·9 : 0·9). The major structural feature appeared to be a β-(1 → 4)-linked D-xylan backbone (possibly containing some 1 → 2 linkages), heavily substituted in the 2-positions by short side-chains of D-xylose (and L-arabinose) and D-glucuronic acid (3). This structure was not common to plant gums but bore some relation to sapote gum from *Sapota achras*.

[47] D. M. W. Anderson and A. C. Munro, *Carbohydrate Res.*, 1969, **11**, 43.
[48] D. M. W. Anderson and A. C. Munro, *Phytochem.*, 1969, **8**, 633.
[49] D. M. W. Anderson and I. C. M. Dea, *Phytochem.*, 1969, **8**, 167.
[50] P. I. Bekker, S. C. Churnas, A. M. Stephen, and G. R. Woolard, *Tetrahedron*, 1969, **25**, 3359.
[51] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1969, **10**, 161.
[52] D. M. W. Anderson, I. C. M. Dea, and A. C. Munro, *Carbohydrate Res.*, 1969, **9**, 363.
[53] A. S. Cerezo, M. Stacey, and J. M. Webber, *Carbohydrate Res.*, 1969, **9**, 505.

-4β-D-Xyl*p*-(1 → 4)-β-D-Xyl*p*-(1 → 4)-β-D-Xyl*p*-(1 → 4 or 2)-β-D-Xyl*p*1 →

2	2	2	2 or 4
↑	↑	↑	↑
1	1	1	1
(L-Ara*p*) D-Xyl*p*	(4-*O*-Me) α-D-G*p*A	4-*O*-Me-α-D-G*p*A	(4-*O*-Me) α-D-G*p*A
4	2	2	
↑	↑	↑	
1	1	1	
D-Xyl*p*	D-Xyl*p*	D-Xyl*p*(L-Ara*p*)	

(3) Average unit of brea gum

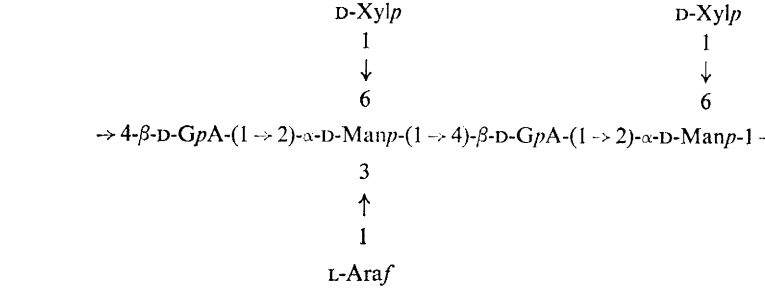
Two distinct polysaccharides have been isolated from *Anogeissus leiocarpus*.[54] Graded precipitation with cetyltrimethylammonium bromide gave a major component leiocarpan A ($[\alpha]_D$ +14°) and a minor component leiocarpan B ($[\alpha]_D$ −5°). Both polymers contained D-glucuronic acid, D-mannose, D-galactose, L-arabinose, and D-xylose although in different proportions. Autohydrolysis of leiocarpan A gave a series of oligosaccharides (4)—(12), whereas more drastic conditions of partial acid hydrolysis resulted in the formation of further quantities of oligosaccharides

(4) β-L-Ara*f*-(1 → 3)-L-Ara
(5) β-L-Ara*p*-(1 → 3)-L-Ara
(6) β-D-Gal*p*-(1 → 3)-D-Gal
(7) β-D-Gal*p*-(1 → 6)-D-Gal
(8) β-D-Gal*p*-(1 → 3)-L-Ara
(9) β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 3)-L-Ara
(10) β-D-G*p*A-(1 → 6)-D-Gal
(11) β-D-G*p*A-(1 → 6)-D-Gal*p*-(1 → 3)-L-Ara
(12) β-D-G*p*A-(1 → 2)-D-Man
(13) β-D-G*p*A-(1 → 2)-D-Man*p*-(1 → 4)-β-D-G*p*A-(1 → 2)-D-Man

(10)—(12) and (13). Partial hydrolysis of leiocarpan B gave a similar series of oligosaccharides (4)—(8), (10), (12), (13). Re-examination of gum ghatti from *A. latifolia* showed that oligosaccharide (13) was also present in partial hydrolysates of this polysaccharide. The structure of the interior chains of leiocarpan A was investigated by methylation of autohydrolysed leiocarpan A followed by reduction and hydrolysis.[55] Further information, obtained by acetolysis of carboxyl-reduced leiocarpan A, suggested that

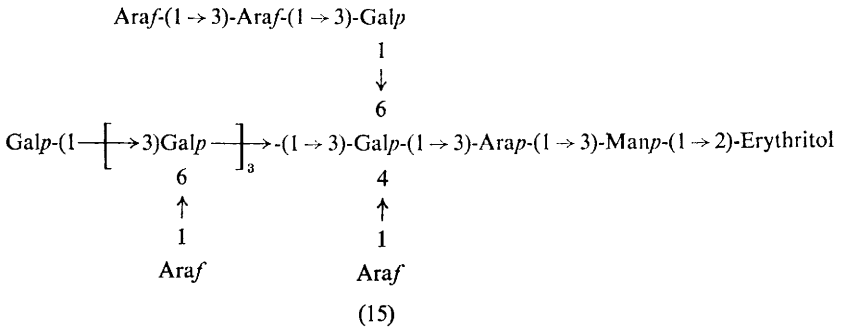
[54] G. O. Aspinall, J. J. Carlyle, J. M. McNab, and A. Rudowski, *J. Chem. Soc.* (C), 1969, 840.
[55] G. O. Aspinall and J. M. McNab, *J. Chem. Soc.* (C), 1969, 845.

the interior chains were composed of alternate 4-*O*-substituted-β-D-glucuronic acid and 2-*O*-substituted-α-D-mannopyranose units. Methylation of intact leiocarpan A, followed by reduction and hydrolysis, gave a complex mixture of products [56] which, together with the isolation of 2-*O*-α-D-mannopyranosyl-D-erythritol from Smith degradation of carboxyl-reduced leiocarpan A, allowed the postulation of a structure (14) for the

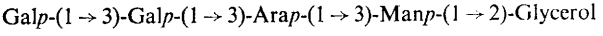


(14)

major part of the polysaccharide. Successive Smith degradations of carboxyl-reduced leiocarpan A in addition gave a series of degraded polysaccharides, leiocarpans A_2—A_5 (15, 16), showing structural features of



(15)



(16)

minor sections of the gum. The erythritol in leiocarpan A_2 (15) represented residual stubs of 4-*O*-substituted glucuronic acid residues in the original polymer, whilst the monomeric and dimeric L-arabinofuranosyl residues were derived from dimer and trimer residues in the original polymer, respectively.

[56] G. O. Aspinall and J. J. Carlyle, *J. Chem. Soc.* (C), 1969, 851.

The mucilage from the bark of *Ulmus fulva* (slippery elm mucilage) contained L-rhamnose, D-galactose, 3-*O*-methyl-D-galactose, and D-galacturonic acid.[57] Oxidation with periodate, borohydride reduction, and partial acid hydrolysis gave a tetrasaccharide (17), whilst more mild acid

3-*O*-Me-D-Gal*p*-(1 → 4)-3-*O*-Me-D-Gal*p*-(1 → 4)-3-*O*-Me-D-Gal*p*-(1 → 4)-L-Rha

(17)

treatment gave a Smith-degraded polysaccharide. In combination with methylation analysis, these results indicated that the structure was more highly branched than was earlier supposed. A major chain of alternate 2-*O*-substituted-L-rhamnopyranose and 4-*O*-substituted-D-galacturonic acid residues was envisaged, substituted with chains of D-galactopyranosyl and 3-*O*-methyl-D-galactopyranosyl groups. A revised estimate of the structure of slippery elm mucilage was made in the light of these findings (18).

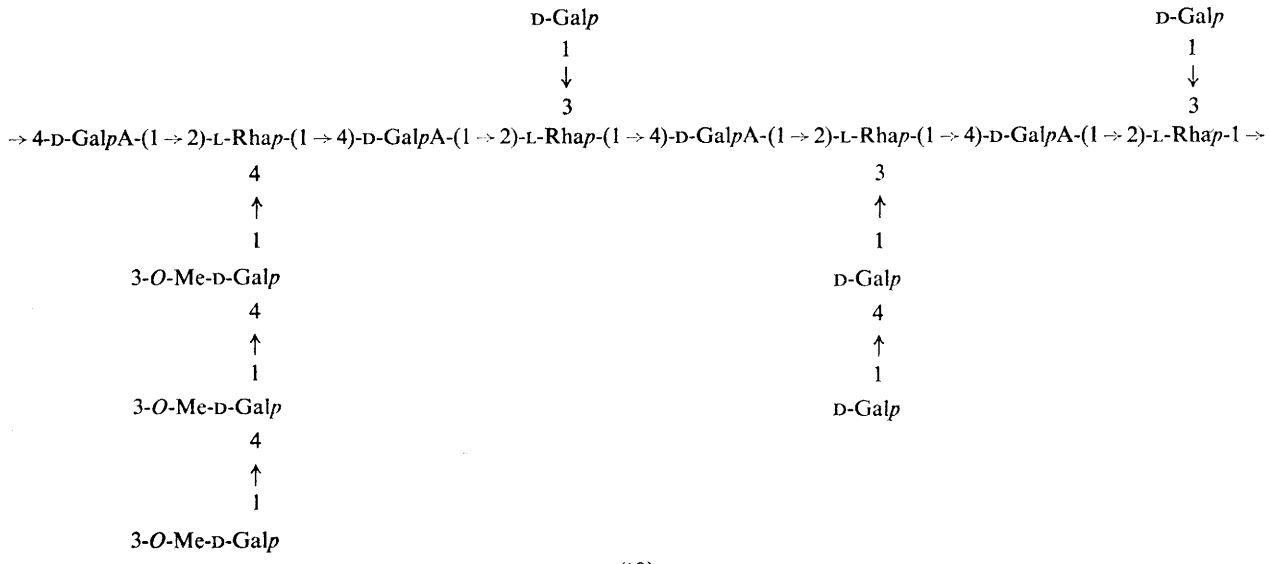
The extracellular polysaccharides of suspension-cultured cells of sycamore (*Acer pseudoplatanus* L.) have been examined.[58] A pectinic acid containing L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid (67%), and structurally similar to other pectinic acids, was isolated. In addition, an arabinogalactan and a fucoxyloglucan were obtained. The arabinogalactan was structurally similar to those from coniferous woods, in that it contained a highly-branched D-galactopyranose framework joined by 1 → 3 and 1 → 6 linkages, with the majority of the side-chains terminated by L-arabinofuranosyl residues (19). As a result of methylation

→ 3-D-Gal*p*-(1 → 3)-D-Gal*p*-(1 → 3)-D-Gal*p*-(1 → 3)-D-Gal*p*-(1 → 3)-D-Gal*p*-1 →		
6	6	6
↑	↑	↑
1	1	1
D-Gal*p*	L-Ara*f*-(1 → 3)-D-Gal*p*	L-Ara*f*-(1 → 3)-D-Gal*p*
6	6	6
↑	↑	↑
1	1	1
L-Ara*f*	D-Gal*p*	D-Gal*p*
5		6
↑		↑
1		1
L-Ara*f*		L-Ara*f*

(19)

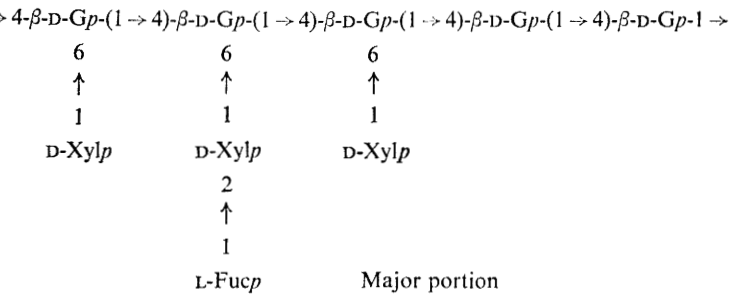
[57] R. J. Beveridge, J. F. Stoddart, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1969, **9**, 429.

[58] G. O. Aspinall, J. A. Molloy, and J. W. T. Craig, *Canad. J. Biochem.*, 1969, **47**, 1063.



(18)

and enzyme degradation studies the fucoxyloglucan was assigned a structure similar to those of the so-called seed 'amyloids'. A cellulose-like (1 → 4)-linked β-D-glucan chain, with side-chains terminated by L-fucopyranosyl and D-xylopyranosyl residues, was postulated (20). The detection



(20)

of minor fragments of 4-substituted chains of D-xylose suggested a more complex structure, but the presence of a D-xylan contaminant could not be excluded in the absence of a clear demonstration of the homogeneity of the polysaccharide.

The electrochemistry[59] and viscosity behaviour[60] of the acidic polysaccharide from the tree *Lannea grandis* have been examined.

The extraction of *O*-acetylated 4-*O*-methyl-D-glucuronoxylans by DMSO from *Castanea sativa* (sweet chestnut) and *Ulmus glabrau* (wych elm), both from green wood and wood exposed to 100% relative humidity, has been studied.[61] Delignification with chlorine–ethanolamine caused a loss of *O*-acetyl groups, the acid chlorite method being preferred, although this caused some conversion to oligomeric *O*-acetylated xylose units during either delignification, or, as a result of glycoside hydrolysis, during incubation. The locations of the *O*-acetyl groups were established by use of phenyl isocyanate as a blocking agent. A mixture of polysaccharides containing xylose, galactose, glucose, and glucuronic acid was extracted with hot water after DMSO extraction.[62] Analysis of these polysaccharides suggested that only the xylans in the green wood of these trees were acetylated, and loss of *O*-acetyl substituents was demonstrated on incubation of the polysaccharides under similar conditions to those employed for the original woods.

[59] S. R. Chaudhuri, B. K. Seal, and S. K. Mukherjee, *J. Indian Chem. Soc.*, 1969, **46**, 153.
[60] S. R. Chaudhuri and S. K. Mukherjee, *J. Indian Chem. Soc.*, 1969, **46**, 109.
[61] G. C. Cochrane, J. D. Grey, and P. C. Arni, *Biochem. J.*, 1969, **113**, 243.
[62] G. C. Cochrane, J. D. Grey, and P. C. Arni, *Biochem. J.*, 1969, **113**, 253.

The 6-kestose, *O*-β-D-fructofuranosyl-(2 → 6)-β-D-fructofuranosyl-α-D-glucopyranoside, has been isolated from the seeds of horse chestnut (*Aesculus hippocastanum* L.).[63]

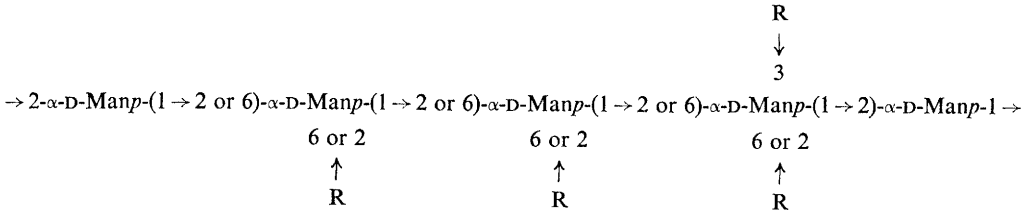
An alkali-soluble galactomannan ($[\alpha]_D$ +79°) has been characterised in extracts of oak lichen (*Evernia prunastri* L.).[64] The polysaccharide contained D-galactose (36%), D-mannose (54%), and D-galacturonic acid (10%). Methylation and periodate oxidation studies indicated that the heteroglycan was a highly-branched acidic galactomannan with a basic skeleton of 1 → 2- and 1 → 6-linked mannan with D-galactopyranosyl and D-galactopyranosyluronic acid terminal residues (21).

Two polysaccharide fractions have been isolated from the seeds of *Cassia absus*, *via* a barium complex.[65] An acetic-acid-insoluble fraction ($[\alpha]_D$ +23·8°) and an acetic-acid-soluble fraction ($[\alpha]_D$ +22·1°) were obtained. Both fractions contained D-mannose, D-galactose, and D-xylose in very similar proportions. The insoluble fraction ($[\alpha]_D$ +23·8°) was essentially a D-galacto-D-mannan (1 : 3), and a representative structure (22) was established on the basis of methylation and periodate oxidation studies.[66] Although similar to other seed galactomannans, the presence of 1,2,3-linked D-mannopyranose branching points was unusual. These branch points have been found in *Trichophyton granulosum* galactomannans.

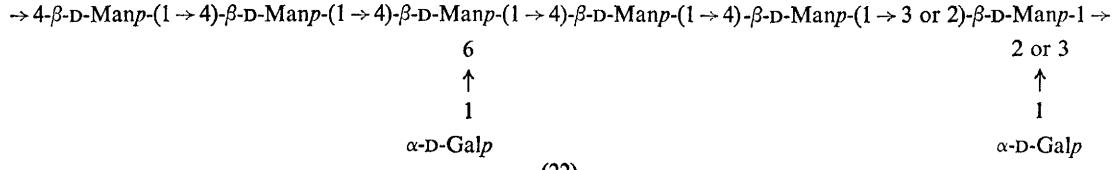
A small proportion of 1,3-substituted D-glucopyranose units were found in the dextran of tibi grain.[67] The polysaccharide {$[\alpha]_D$ +206° (NaOH)}, had an average $\overline{DP}$ of 3630 and average chain length of 18. Methylation gave evidence for both branching through C-3 and C-6, and single substitution at C-3. Smith degradation gave glycerol, erythritol, and 1-*O*-α-D-glucopyranosyl-glycerol (56 : 1 : 6).

The hemicelluloses of oat plants, *Avena sativa* var. Blenda, have been examined with respect to growth and distribution. The molecular composition was considered in terms of polymolecularity, polydispersity, and polydiversity for use in determining the relationship between hemicellulose composition and maturity.[68] Fractionation procedures were established, and high recoveries of well-fractionated hemicellulosic material obtained by DEAE-cellulose chromatography. The total hemicellulose extracted with potassium hydroxide from the holocellulose of the leaves of young plants contained galactose, glucose, arabinose, and xylose (2·8 : 11·4 : 10 : 23·5) and small amounts of uronic acid.[69] The water-soluble fraction was fractionated to give a non-glucose-containing polysaccharide (15%). This

[63] W. Kahl, A. Roszkowski, and A. Zurowska, *Carbohydrate Res.*, 1969, **10**, 586.
[64] V. M. Micovic, M. Hranisavljevic-Jakovljevic, and J. Miljkovic-Stojanovic, *Carbohydrate Res.*, 1969, **10**, 525.
[65] V. P. Kapoor and S. Mukherjee, *Current Sci.*, 1969, **38**, 38.
[66] V. P. Kapoor and S. Mukherjee, *Canad. J. Chem.*, 1969, **47**, 2883.
[67] M. Horiberger, *Carbohydrate Res.*, 1969, **10**, 379.
[68] J. G. S. Reid and K. C. B. Wilkie, *Phytochem.*, 1969, **8**, 2045.
[69] J. G. S. Reid and K. C. B. Wilkie, *Phytochem.*, 1969, **8**, 2053.


R
3
→ 2-α-D-Manp-(1 → 2 or 6)-α-D-Manp-(1 → 2 or 6)-α-D-Manp-(1 → 2 or 6)-α-D-Manp-(1 → 2)-α-D-Manp-1 →
6 or 2
6 or 2
6 or 2
R
R
R
R = β-D-Galp-1 → OR D-GalpA-1 →

(21)


→ 4-β-D-Manp-(1 → 4)-β-D-Manp-(1 → 4)-β-D-Manp-(1 → 4)-β-D-Manp-(1 → 4)-β-D-Manp-(1 → 3 or 2)-β-D-Manp-1 →
6
2 or 3
1
1
α-D-Galp
α-D-Galp

(22)

galactoarabinoxylan (2·8 : 10 : 18·5) was structurally similar to other land-plant xylans in having a backbone of 1 → 4-linked β-D-xylopyranose units and terminal arabinofuranosyl residues. An unusual feature was the presence of terminal galactopyranosyl residues. Glucuronic acid, 4-*O*-methyl-glucuronic acid, a glucuronosylxylose, and an aldotriouronic acid were isolated from acid hydrolysates. Isolation of total hemicelluloses and subsequent analysis of purified fractions showed that it was inappropriate to study the effect of growth on hemicellulosic composition solely by consideration of pure hemicelluloses.[70] In any one part of the plants studied, there was an increase in the percentage of xylose, and a decrease in that of glucose with increasing maturity. The changes in composition were related to the three polysaccharides known to be present; an arabinoxylan, an acidic galactoarabinoxylan, and a non-cellulosic glucan.

The biosynthesis of a glucomannan has been described, employing an enzyme system from mung-bean seedlings.[71] Mannose was incorporated from GDP-D-[^{14}C]mannose and glucose from GDP-D-[^{14}C]glucose into an alkali-insoluble glucomannan. Incorporation of D-[^{14}C]glucose was dependent on GDP-D-mannose and gave a polysaccharide with the label solely in the glucose residues. Incorporation of D-[^{14}C]mannose resulted in some labelling in the D-glucose residues in the polymer and the incorporation was inhibited by GDP-D-glucose. The polymer appeared to be a β-(1 → 4)-linked glucomannan with three or four mannose residues per glucose residue.

The effect of enzymolysis of the F-1 β-D-glucan of naked barley with a laminarase and a cellulase has been reported.[72]

The water-soluble polysaccharides from leaves of *Tussilago farfara* L. (coltsfoot), an important species in traditional pharmacy, have been studied.[73] The crude polysaccharide mixture contained D-galacturonic acid, D-glucose, D-galactose, L-arabinose, D-xylose, D-ribose, L-rhamnose, and traces of 3-*O*-methyl sugars. Five acidic fractions were obtained by anion-exchange chromatography, which were characterised by their varying sugar composition and increasing D-galacturonic acid content. The major fraction appeared to be a typical pectin.

A number of reports have appeared concerning the isolation, characterisation, and enzymic degradation of pectic substances. Methylation analysis of the pectic polysaccharide from cotyledons of white mustard indicated that the structural units present were similar to those in the well-known polysaccharide from mature tissues, but of strikingly different proportions.[74] The linkages of the D-galacturonic acid residues were established by the isolation of 2,3-di-*O*-methyl-D-[6-^{3}H]galactose and

[70] J. G. S. Reid and K. C. B. Wilkie, *Phytochem.*, 1969, **8**, 2059.
[71] A. D. Elbein, *J. Biol. Chem.*, 1969, **244**, 1608.
[72] O. Igarashi, M. Fujimaki, and Y. Sakurai, *J. Ferm. Technol.*, 1969, **47**, 456.
[73] E. Haaland, *Acta Chem. Scand.*, 1969, **23**, 2546.
[74] D. A. Rees and N. J. Wight, *Biochem. J.*, 1969, **115**, 431.

2-*O*-methyl-D-[6-^{3}H]galactose after sodium-[^{3}H]borohydride reduction of the methylated polysaccharide. These results suggested a galacturonorhamnan backbone with arabinan side-chain and single xylose substituents. It was concluded that uninterrupted and unbranched galacturonan segments could not contribute to the cohesion of these walls, and it was suggested that this correlated with a function of the wall matrix to hydrate and permit readjustment of structural units or wall surfaces during germination.

Partial hydrolysis of zosterne, the pectic acid of Zosteraceae, has shown it to contain linear segments of 1 → 4- (or possibly 1 → 5-) linked D-galacturonic acid residues.[75] A similar skeleton was proposed for the pectin from roots of *Panax ginseng* C. A. Mey.[76] This polysaccharide contained D-galactose and L-arabinose, in addition to D-galacturonic acid, as major components, and D-xylose, L-rhamnose, and an unidentified sugar as minor components. Pectinase digestion gave a neutral polysaccharide in which L-arabinofuranosyl residues apparently occurred in exterior chains. Partial acid hydrolysis of this polysaccharide afforded a branched galactan, containing chains of 1 → 3- and 1 → 6-linked D-galactopyranose units, and an acidic heteropolysaccharide containing sequences involving D-galactopyranose residues and others involving D-galacturonic acid, D-xylose, and L-rhamnose residues.

The pectic substances of pigmented onion skins have been characterised and the products of acid decomposition studied.[77] A scheme has been devised for the determination of free amino-acids, organic non-amino-acids, sugars, and nitrogen in plant materials of the pectin type.[78]

The distribution of free carboxy-groups in the pectins produced by esterification of pectic acid and pectinic acid by methanol have been determined.[79] The pectins were characterised by their degree of esterification, polyuronide content, viscosity, molecular weight, and stability constants of the calcium pectinates. The distribution of free and esterified carboxy-groups was approximately statistical in compounds with easily accessible carboxy-groups, *viz.* pectic acid in a gel form or pectinic acid with DE 25%, but compacted powdered pectic acid aggregates gave groupwise arrangements. An ion-exchange technique has been described for the isolation of oligo-D-galacturonic acids of DP 2—8 from enzymic cleavage of pectic acids.[80]

Part of the acidic cell walls of leaves from *Cyclea barbata* and other species dissolved on disintegration with cold water.[81] This highly esterified

[75] R. G. Ovodova and Yu. S. Ovodov, *Carbohydrate Res.*, 1969, **10**, 387.
[76] T. F. Solov'eva, L. V. Arsenyuk, and Yu. S. Ovodov, *Carbohydrate Res.*, 1969, **10**, 13.
[77] A. F. Abdel-Fattah and M. Edrees, *J. Chem. U.A.R.*, 1968, **11**, 383.
[78] K. Salminen and P. Koivistoinen, *Acta Chem. Scand.*, 1969, **23**, 999.
[79] R. Kohn and J. Furda, *Coll. Czech. Chem. Comm.*, 1969, **34**, 641.
[80] C. W. Nagel and T. M. Wilson, *J. Chromatog.*, 1969, **41**, 410.
[81] P. Kooiman, *J. Sci. Food Agric.*, 1969, **20**, 18.

pectin was gradually de-esterified on standing, with resultant gelation, as a result of pectin esterase action.

An unusually acid-stable *endo*-polygalacturonase has been purified and characterised from the basidomycete *Corticium rolfsii*.[82] The differences observed in the action pattern of *endo*-polygalacturonases produced *in vivo* and *in vitro* by *Penicillium expansum* were attributed to changes brought about during isolation of the enzymes.[83] The enzyme extracted from apples gave a tetragalacturonic acid from pectic acid, whilst the enzyme from a culture filtrate gave galacturonic acid.

A fungal *exo*-polygalacturonase, purified from *endo*-activity, only degraded pectic acid to the extent of 42%, and this was considered due to obstruction as a result of the presence of neutral sugar components.[84] Three *exo*-polygalacturonase fractions were isolated from *Aspergillus niger*[85] and one enzyme was shown to be responsible for the degradation of both 4-*O*-α-D-galactopyranosyluronic acid-D-galacturonic acid and 2-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galacturonic acid.[86,87] The *exo*-polygalacturonase from *Erwinia aroideae* appeared to remove digalacturonic acid successively from the non-reducing terminus of pectic acid.[88] A study of a particulate enzyme system from mung-bean shoots demonstrated that methylation proceeded by transmethylation directly onto the polymeric molecule, and not *via* intermediate nucleotide galacturonic acids.[89]

A branched L-arabinan has been purified from crude beet arabinan and degraded by an α-L-arabinofuranosidase from *A. niger* to give a linear α-L-arabinan.[90] Periodate oxidation data was consistent with the presence of only 1 → 5 inter-unit linkages. This *exo*-enzyme also removed the arabinofuranose residues in a wheat flour L-arabino-D-xylan. The water-insoluble portion of the pentosan from Durum wheat endosperm was shown to be an arabinoxylan.[91] Two-thirds of the xylose residues in a β-(1 → 4)-D-xylopyranosyl backbone were branched at either the 3 position or the 2 and 3 positions, and they were substituted with L-arabinofuranosyl side-chains. Branches occurred predominantly on alternating D-xylose units. A separate study[92] showed that the arabinoxylan of wheat flour was essentially a random coil in solution, although somewhat rigid and extended as a result of the short labile arabinose side-chains. The general characterisation of wheat flour pentosans has been reported.[93]

[82] A. Kaji and T. Okada, *Arch. Biochem. Biophys.*, 1969, **131**, 203.
[83] T. R. Swinbarne and M. E. Corden, *J. Gen. Microbiol.*, 1969, **55**, 75.
[84] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. (Japan)*, 1969, **43**, 67.
[85] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. (Japan)*, 1969, **43**, 77.
[86] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. (Japan)*, 1969, **43**, 85.
[87] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. (Japan)*, 1969, **43**, 139.
[88] C. Hatanaka and J. Ozawa, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 116.
[89] H. Kauss and A. L. Swanson, *Z. Naturforsch.*, 1969, **24B**, 28.
[90] K. Tagawa and A. Kaji, *Carbohydrate Res.*, 1969, **11**, 293.
[91] D. G. Medcalf and K. A. Gilles, *Cereal Chem.*, 1968, **45**, 550.
[92] E. W. Cole, *Cereal Chem.*, 1969, **46**, 382.
[93] F. M. Lin and Y. Pomeranz, *J. Food Sci.*, 1969, **33**, 599.

The isolation and characterisation of acid-hydrolysis products of konjac mannan have been described.[94]

The trisaccharide fraction of several Liliaceous species bulbs contained 1^F-β-D-fructofuranosyl-sucrose and 6^G-β-D-fructofuranosyl-sucrose but not 6^F-β-D-fructofuranosyl-sucrose.[95]

The biosynthesis of starch has been reviewed.[96] An amylopectin-type polysaccharide was synthesised in a two-enzyme cell-free system.[97] The combined action of a highly purified α-D-glucan-ADP-glucosyl transferase and a potato α-D-glucan branching transferase synthesised a polysaccharide similar to native amylopectin in its iodine-complex spectral characteristics, its β-amylolysis limit, and basal chain length. The action pattern of a *trans*-maltodextrinylase (D-enzyme) from potato has been determined.[98] Maltosyl was transferred more rapidly than larger groups, and the rate of transfer to glucose was equal in the case of malto-triose, -pentaose, and -hexaose. Empirical rules were suggested that prohibited the transfer of groups which necessitated the cleavage of either the non-reducing or the penultimate units from the reducing end linkages. Thus maltosyl could not be transferred from maltotetraose. The inhibition of potato phosphorylase by donor substrate analogues has been studied.[99] Kinetic studies indicated that D-glucose, 2-deoxy-D-*arabino*-hexose, 3-*O*- and 6-*O*-methyl-D-glucose behaved as competitive inhibitors of enzymic synthesis. The differential behaviour of malto-triose, -tetraose, and -hexaose as primers in the enzymic synthesis of amylose with potato phosphorylase has also been studied.[100] In each case a multi-chain mechanism was operative, but whilst the polymer from malto-tetraose and -hexaose exhibited a Poisson molecular weight distribution, that from maltotriose was composed of a mixture of a uniform high-molecular-weight polymer and a low-molecular-weight fraction with a broad distribution. These differences were shown to be a result of the maltotriose starting reaction being some four hundred times slower than the growing reaction, whilst the two reactions with the tetramer and hexamer were of comparable rate. Amylo-1,6-glucosidase-oligo-1,4–1,4-transferase, the glycogen phosphorylase limit debranching system, has been purified and characterised,[101] and the kinetics of a maltodextrin phosphorylase examined.[102]

[94] K. Kato and K. Matsuda, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 1446.
[95] H. Hammer, *Acta Chem. Scand.*, 1969, **23**, 3268.
[96] R. Geddes, *Quart. Rev.*, 1969, **23**, 57.
[97] A. Doi, *Biochim. Biophys. Acta*, 1969, **184**, 477.
[98] G. Jones and W. J. Whelan, *Carbohydrate Res.*, 1969, **9**, 483.
[99] J. Hollo, E. Laszlo, and A. Hoschke, *Carbohydrate Res.*, 1969, **10**, 49.
[100] B. Pfannemuller and W. Burchard, *Die Makromol. Chem.*, 1969, **121**, 1.
[101] T. E. Nelson, E. Kolb, and J. Larner, *Biochemistry*, 1969, **8**, 1419.
[102] J. Chao, G. F. Johnson, and D. J. Graves, *Biochemistry*, 1969, **8**, 1459.

4
Microbial Polysaccharides

Bacterial Polysaccharides

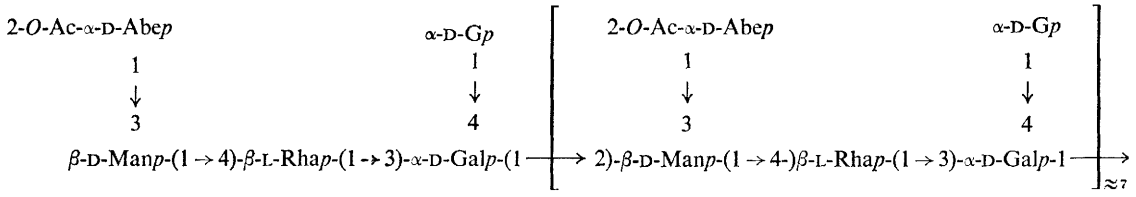
Further studies have been made on the structural chemistry of the lipopolysaccharides of the enterobacteria particularly with respect to their immunochemical relationships. Lindberg's group have continued studies on the *Salmonella* species. The O-specific side-chains of the lipopolysaccharide of *S. typhimurium* LT2 have been studied to assess whether the observed differences between the *S. typhimurium* 395MS lipopolysaccharide and the rest of the group B polymers were a general feature.[103] Methylation analysis of the intact LT2 polysaccharide and its partial acid hydrolysis products revealed that it was essentially similar to the 395MS polysaccharide (23). The position of the *O*-acetyl group was determined by acetal formation with methylvinylether, deacetylation, methylation, and deacetalation, and found to be at the C-2 position of approximately 50% of the terminal abequopyranose units. A higher proportion of the D-galactopyranose residues in the LT2 polysaccharide were substituted by α-D-glucopyranosyl residues than in the 395MS polymer.

The lipopolysaccharide of *S. bredeney* (1, 4, 12, O factors) contained D-glucose, D-galactose, D-mannose, L-rhamnose, and abequose (10 : 25 : 23 : 22 : 20) and no *O*-acetyl groups.[104] Methylation of the original polysaccharide and its partial acid hydrolysate, which removed the abequose residues and broke some of the rhamnosyl linkages, allowed the formulation of a probable repeating sequence (24). The absence of the *O*-acetyl groups was in agreement with the absence of O factor 5, and the suggestion that the O factor 1 was associated with the presence of terminal α-D-glucopyranosyl residues attached to the 6-position of the D-galactose residues was substantiated by this structure. This structure, when taken with others for the *S. typhimurium* lipopolysaccharides, suggested a common repeating unit (25) for the O-specific chains to which various substituents were attached for individual specificities.[104]

Study of the lipopolysaccharides from *S. typhi* (1.S.59) and *S. enteritidis* (1.S.64) serotype D_1 revealed a close chemical similarity between the

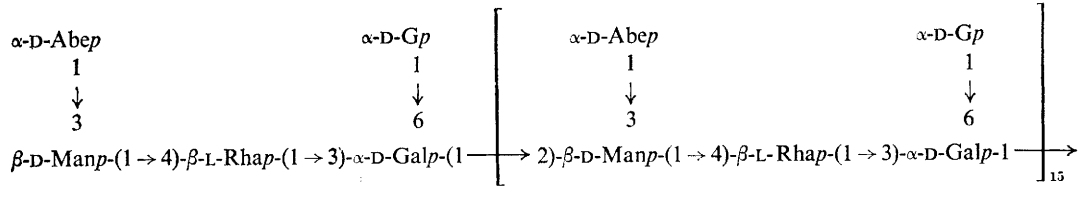
[103] C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Carbohydrate Res.*, 1969, **9**, 237.

[104] C. G. Hellerqvist, O. Larm, B. Lindberg, T. Holme, and A. A. Lindberg, *Acta Chem. Scand.*, 1969, **23**, 2217.



Salmonella typhimurium LT2 Lipopolysaccharide O-specific chains

(23)



Salmonella bredeney Lipopolysaccharide O-specific chains

(24)

$$\begin{array}{c}\alpha\text{-D-Abe}p\\ 1\\ \downarrow\\ 3\end{array}$$

$$\rightarrow 2\text{-}\beta\text{-D-Man}p\text{-}(1\rightarrow 4)\text{-}\beta\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}1\rightarrow$$

(25)

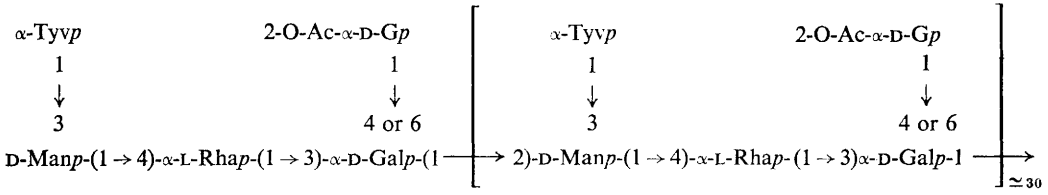
O-specific chains in serotypes B and D.[105] This lipopolysaccharide contained D-mannose, D-galactose, D-glucose, L-rhamnose, tyvelose, and *O*-acetyl groups. The position of substitution of the constituent units was established by direct methylation, the mutual order of units by methylation of partial acid hydrolysates, and the position of *O*-acetylation by methylation after protection by acetal formation (methylvinylether) and deacetylation, allowing a probable repeating sequence to be postulated (26). The major difference of structure between serotype B and serotype D lipopolysaccharides was the presence of terminal abequose (B) or tyvelose (D) residues. Some polysaccharides in the serogroup B had *O*-acetyl substituents on the abequose residues, whilst in serogroup D these substituents were on the terminal D-glucopyranosyl residues, in both cases substitution being at C-2.

The M-antigen of *S. typhimurium* 395MRO-M contained D-glucose, D-galactose, L-fucose, and D-glucuronic acid.[106] Methylation gave, *inter alia*, 2,6-di-*O*-methyl-D-galactose and 2-*O*-methyl-L-fucose, indicative of a number of branch points. Since no end-groups were revealed by the methylation analysis the presence of an acetal or ketal was suggested. Prior treatment of the polysaccharide with acid resulted in the formation of 2,3,4,6-tetra-*O*-methyl-D-galactose at the expense of 2,6-di-*O*-methyl-D-galactose on methylation. An ethylidene group was indicated by the isolation of acetaldehyde as its 2,4-dinitrophenylhydrazone. A probable repeating sequence was postulated (27).

Although the core region of the lipopolysaccharide from *S. friedenau* T_1 was similar to that of other *Salmonella* lipopolysaccharides, the T_1-specific side-chains showed distinct differences.[107] The T_1 chains were composed of almost equal amounts of β-D-ribofuranose and β-D-galactofuranose residues. The ribofuranose units were 2-*O*-substituted whilst the galactofuranose residues were 3-*O*-, 6-*O*-, or 3,6-di-*O*-substituted. Both constituents appeared as terminal residues (28).

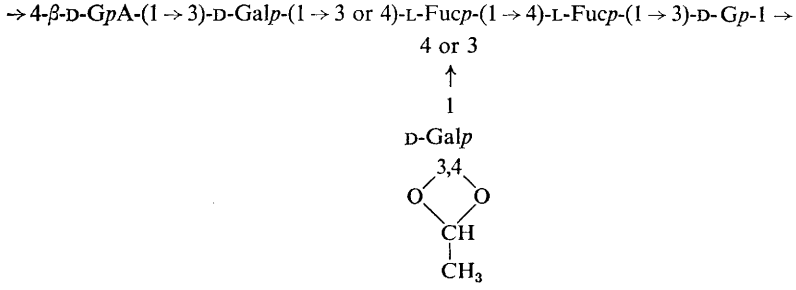
Growth of the amoeba *Dictyostelium discoideum* on *Salmonella london* resulted in the secretion into the medium of a degraded, water-soluble,

[105] C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Acta Chem. Scand.*, 1969, **23**, 1588.
[106] P. J. Garegg, B. Lindberg, T. Onn, and T. Holme, *Acta Chem. Scand.*, 1969, **23**, 2194.
[107] M. Berst, C. G. Hellerqvist, B. Lindberg, O. Luderitz, S. Svensson, and O. Westphal, *European J. Biochem.*, 1969, **11**, 353.



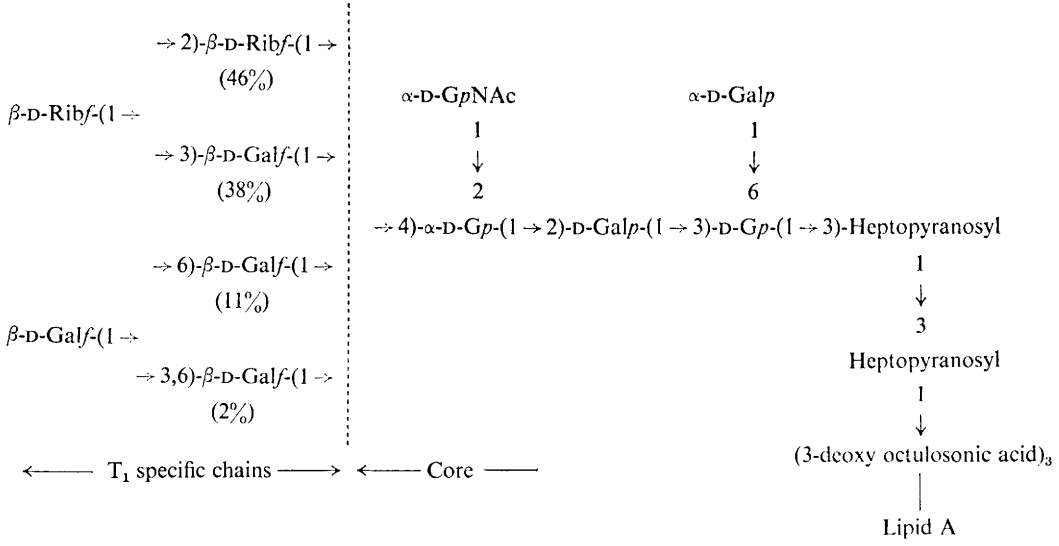
Salmonella typhi serogroup D_1 lipopolysaccharide O-specific chains

(26)



M-antigen of *Salmonella typhimurium* 395MRO-M

(27)

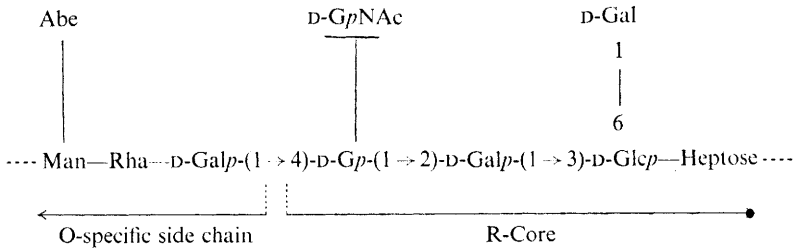


Salmonella friedenau T_1 Lipopolysaccharide

(28)

form of the bacterial lipopolysaccharide.[108] The degraded polymer contained all the constituent sugars of the parent lipopolysaccharide, including the 2-acetamido-2-deoxy-D-glucose of the lipid A component, as well as *O*-acetyl groups attached to the D-galactose units in the O-specific chains. The polysaccharide was completely devoid of ester- and amide-linked long-chain fatty acids present in the lipid A component of the parent lipopolysaccharide. The degraded polymer retained its serological O-specificity.

The linkage between the O-specific side-chains and the R core in the lipopolysaccharides of *S. typhimurium* has been deduced using lipopolysaccharides of wild type (S form).[109] Smith degradation gave, *inter alia*, the oligosaccharide D-galactopyranosyl-(1 → 4)-D-glucopyranosyl-(1 → 2)-glyceraldehyde. Degradation of a polysaccharide prepared by an *in vitro* synthesis from O-chains with D-galactose-[^{14}C] only at the reducing terminus gave this oligosaccharide with a D-galactose-[^{14}C] residue at the non-reducing terminus. The galactose residue of the O-specific chains were not therefore attached to the terminal 2-acetamido-2-deoxy-D-glucose unit of the R core, but to an interior D-glucose residue (29).



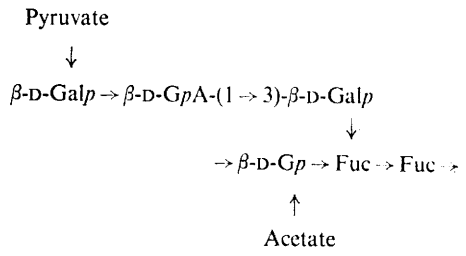
(29)

The polysaccharides of virulent and avirulent strains of *S. flexneri* have been examined.[110]

The backbone of the lipid A component of *Salmonella* R mutants contained phosphorylated (2-acetamido-2-deoxy-D-glucosyl)-2-acetamido-2-deoxy-D-glucose units, probably β-(1 → 6) linked, to which a trisaccharide of 3-deoxy-octulosonic acid residues was linked ketosidically.[111] This pentasaccharide appeared to be a common structural feature in many Enterobacteriaceae. The specificity of the lipopolysaccharide acceptor sites and the role of phospholipid have been studied for a UDP-galactose: lipopolysaccharide-α-3-galactosyltransferase.[112, 113]

[108] D. Malchow, O. Luderitz, B. Kickhofen, and O. Westphal, *European J. Biochem.*, 1969, **7**, 239.
[109] H. Nkaido, *J. Biol. Chem.*, 1969, **244**, 2835.
[110] J. Pogonowska-Goldhar and H. Stypulkowska-Misiurewicz, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1968, **16**, 751.
[111] J. Gmeiner, O. Luderitz, and O. Westphal, *European J. Biochem.*, 1969, **7**, 370.
[112] A. Endo and L. Rothfield, *Biochemistry*, 1969, **8**, 3500.
[113] A. Endo and L. Rothfield, *Biochemistry*, 1969, **8**, 3508.

Colanic acid, a common exopolysaccharide in Enterobacteriaceae, from *S. typhimurium*, *Aerobacter cloacae*, and *Escherichia coli*, contained D-glucose, D-galactose, L-fucose, D-glucuronic acid, acetate, and pyruvate (1 : 1·8 : 1·9 : 1 : 1 : 1).[114] Periodate oxidation of native and deacetylated colanic acid indicated that the D-glucose residues were the site of *O*-acetylation and the D-galactose residues the site of the pyruvic acid ketal substituents. The isolation of a number of oligosaccharides from partial hydrolysates of colanic acid and carboxy-reduced colanic acid (sodium borohydride[^{3}H]) enabled a sequence of units to be postulated (30).

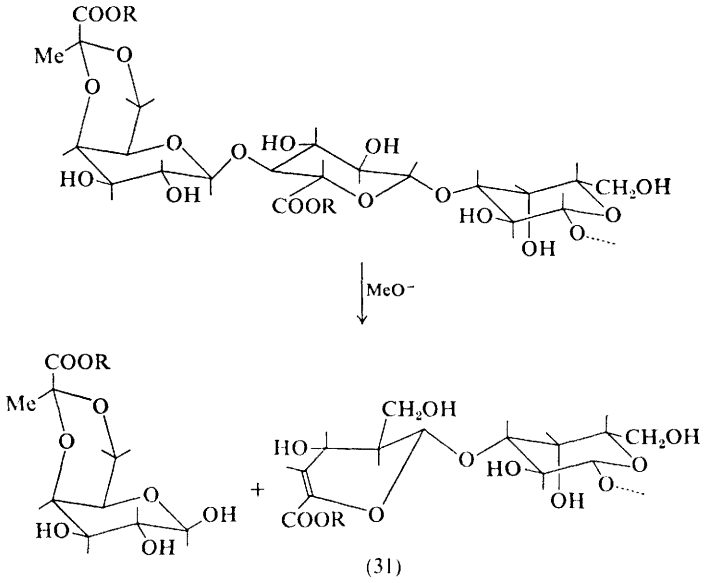


(30)

Methylation of the colanic acid from *E. coli* K12 followed by methanolysis gave, *inter alia*, a methyl glycoside of 4,6-*O*-(1′-methoxycarbonylethylidene)-2,3-di-*O*-methyl-D-galactose.[115] On treatment with base under anhydrous conditions the hydroxypropyl ester of colanic acid underwent a β-elimination reaction at the uronate residues to release a 4,6-*O*-(1′-alkoxycarbonylethylidene)-D-galactose (31) (Scheme 3). Together with the identification of 3-*O*-(D-glucopyranosyluronic acid)-D-galactose as a partial acid hydrolysis product, this suggested that most, if not all, of the side-chains of colanic acid were composed of *O*-[4,6-*O*-(1′-carboxyethylidene)-D-galactopyranosyl]-(1 → 4)-*O*-(D-glucopyranosyluronic acid)-(1 → 3)-D-galactopyranosyl residues. The production of colanic acid by various strains from *Salmonella*, *Escherichia*, and *Aerobacter* under different growth conditions has been surveyed.[116]

Structural analysis of the lipopolysaccharides of *E. coli* strains from A295b (serotype 08: K42(A): H$^-$) and E56b (serotype 08: K27(A): H$^-$) showed that the O-specific chains were identical but that the core structures were different.[117] Thus, unlike the lipopolysaccharides from *Salmonella* and *Shigella* which each possessed common cores, various strains of *E. coli* had different lipopolysaccharide core structures.

[114] I. W. Sutherland, *Biochem. J.*, 1969, **115**, 935.
[115] C. J. Lawson, C. W. McCleary, H. I. Nakada, D. A. Rees, I. W. Sutherland, and J. F. Wilkinson, *Biochem. J.*, 1969, **115**, 947.
[116] W. D. Grant, I. W. Sutherland, and J. F. Wilkinson, *J. Bacteriol.*, 1969, **100**, 1187.
[117] G. Schmidt, B. Jann, and K. Jann, *European J. Biochem.*, 1969, **10**, 501.



(31)

Scheme 3

2-Amino-2-deoxy-D-mannuronic acid has been found as a major component in the acidic polysaccharide from *E. coli* 014: K7: H$^-$ and 07: K7: H4.[118]

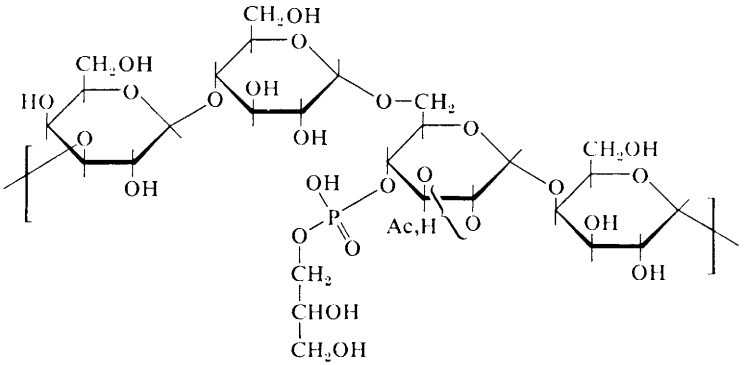
The formation of nucleoside diphosphate sugar precursors of the K27 antigen of *E. coli* 08: K27(A): H$^-$ has been described.[119]

A group of mutants from *Klebsiella aerogenes* strains synthesised much less polysaccharide slime or capsule than the parent bacteria on incubation at low temperature, but similar amounts at 37°.[120]

Comparative analysis of the O-specific side-chains of *Shigella flexneri* serotypes 1a and 2a revealed a tetrasaccharide repeating unit, differing in the site of substitution of a terminal α-D-glucopyranosyl residue.[121] The common group antigen immunodominant sugar was rhamnose. A more complete relationship between the structure of the O-specific side-chains and the immunological reactions was later established.[122] Two common unbranched structures of Y variants, Y_1 and Y_2, were precursors of the different serotypes which were formed by incorporation of side-chains of D-glucose in a position characteristic of each serotype (32) (see p. 224–225).

A repeating sequence (33) for the type-specific polysaccharide of *Pneumococcus* type 11A(U.S. 43) has been deduced.[123] This polysaccharide

[118] H. Mayer, *European J. Biochem.*, 1969, **8**, 139.
[119] A. C. Olson, G. Schmidt, and K. Jann, *European J. Biochem.*, 1969, **11**, 376.
[120] M. Norval and I. W. Sutherland, *J. Gen. Microbiol.*, 1969, **57**, 369.
[121] D. A. R. Simmons, *Biochem. J.*, 1969, **114**, 34P.
[122] D. A. R. Simmons, *European J. Biochem.*, 1969, **11**, 554.
[123] D. A. Kennedy, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, 1969, **115**, 37.



(33)

contained D-glucose, D-galactose, glycerol, phosphate, and *O*-acetyl groups (2 : 2 : 1 : 1 : 2). Removal of the *O*-acetyl groups with ammonia destroyed the ability of the polysaccharide to precipitate anti-type 11A serum. Reduction with sodium borohydride, followed by alkaline hydrolysis, gave a phosphate-free polysaccharide whose structure was established by methylation and periodate oxidation. Periodate oxidation of the original polysaccharide and de-*O*-acetylated polysaccharide gave further data, although the location of one *O*-acetyl group was not established. This structure was similar to that established for type 18 polysaccharide in which the *O*-acetyl was again immunologically important. Smith degradation of type 11A polysaccharide gave two tetrasaccharides, one of which appeared to be branched from periodate oxidation data. It was concluded that this was most likely to be an artefact arising from cyclic acetal formation with glycolaldehyde, since all other evidence indicated the absence of branching.

A pentasaccharide corresponding to the dephosphorylated repeating unit of *Pneumococcus* type 29 type-specific polysaccharide was obtained by alkaline hydrolysis and enzymic dephosphorylation.[124] Acid hydrolysis of the intact polysaccharide gave D-galactose, 2-amino-2-deoxy-D-galactose, anhydro-ribitol, ribitol phosphate, ribitol, 2-acetamido-2-deoxy-D-galactose phosphate, and inorganic phosphate. Deamination with nitrous acid gave 2,5-anhydro-D-talose and a tetrasaccharide. This evidence, together with data from periodate oxidation and from methylation, enabled a partial structure (34) to be formulated.

Methyl-3-*O*-β-D-galactofuranosyl-α-D-glucopyranoside has been synthesised but did not inhibit the homologous precipitin reaction of type 34 polysaccharide with rabbit anti-pneumococcal type 34 serum.[125] Since

[124] E. VenkataRao, M. J. Watson, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, 1969, **111**, 547.

[125] C. P. J. Glaudemans, *Carbohydrate Res.*, 1969, **10**, 213.

Y_1 → 6-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-(1 → 6)-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-1 →

1a → 6-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-(1 → 6)-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-1 →
4 4
↑ ↑
1 1
α-D-G*p* α-D-G*p*

Y_2 → 4-D-G*p*NAc-(1 → 3)-Rha*p*-(1 → 4)-Rha*p*-(1 → 4)-D-G*p*NAc-(1 → 3)-Rha*p*-(1 → 4)-Rha*p*-1 →

4a → 4-D-G*p*NAc-(1 → 3)-Rha*p*-(1 → 4)-Rha*p*-(1 → 4)-D-G*p*NAc-(1 → 3)-Rha*p*-(1 → 4)-Rha*p*-1 →
6 6
↑ ↑
1 1
α-D-G*p* α-D-G*p*

2a → 6-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-(1 → 6)-D-G*p*NAc-(1 → 2)-Rha*p*-(1 → 4)-Rha*p*-1 →
4 4
↑ ↑
1 1
α-D-G*p* α-D-G*p*

3a $\rightarrow$ 4-D-G*p*NAc-(1 $\rightarrow$ 3)-Rha*p*-(1 $\rightarrow$ 4)-Rha*p*-(1 $\rightarrow$ 4)-D-G*p*NAc-(1 $\rightarrow$ 3)- Rha*p*-(1 $\rightarrow$ 4)-Rha*p*-1 $\rightarrow$

2 2

$\uparrow$ $\uparrow$

1 1

O-Ac-α-D-G*p* *O*-Ac-α-D-G*p*

5 $\rightarrow$ 4-D-G*p*NAc-(1 $\rightarrow$ 3)-Rha*p*-(1 $\rightarrow$ 4)-Rha*p*-(1 $\rightarrow$ 4)-D-G*p*NAc-(1 $\rightarrow$ 3)- Rha*p*-(1 $\rightarrow$ 4)-Rha*p*-1 $\rightarrow$

3 3

$\uparrow$ $\uparrow$

1 1

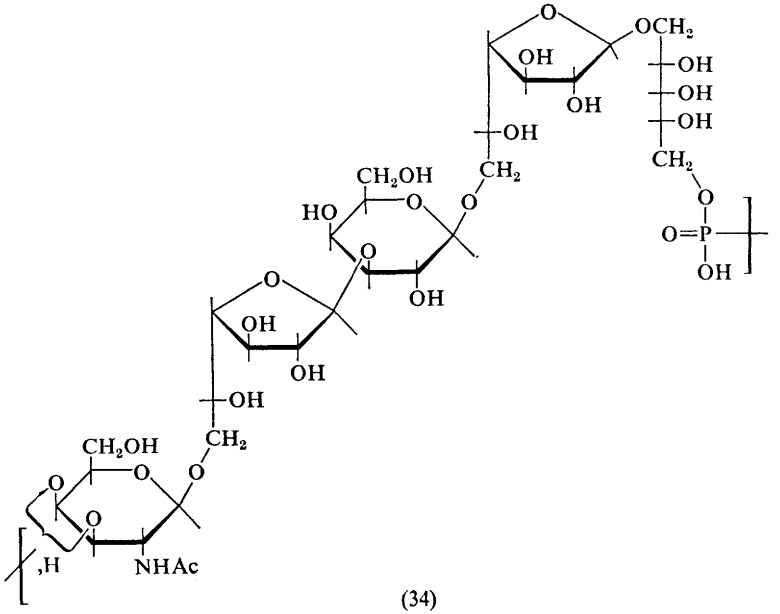
α-D-G*p* α-D-G*p*

Comparison of *Shigella flexneri* O-antigens.

Y_1 structure is a cryptic situation in serotypes 1a and 2a, whilst

Y_2 structure is a cryptic situation in serotypes 3a, 4a and 5.

(32)



(34)

this was the portion of the type 34 polysaccharide to which the *O*-acetyl group was attached, it confirmed the immunodominance of 6-*O*-acetyl-β-D-galactofuranosyl residue in this polysaccharide.

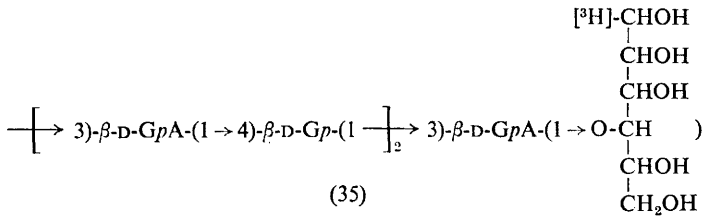
Some structural features of the type-specific polysaccharide of *Pneumococcus* type 19 have been established.[126]

The inhibition of precipitin reactions in the *Pneumococcus* type 2 and type 3 systems by various aldobiuronates has been studied.[127] Since isomaltobiuronic acid was, after D-glucuronic acid, the most effective inhibitor of the type 2–anti-type 2 system it was concluded that isomaltobiuronosyl residues occur as terminal groups in the type-specific polysaccharide of *Pneumococcus* type 2.

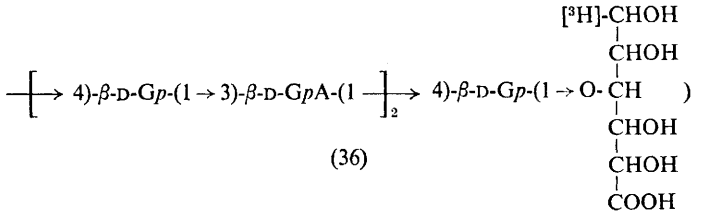
The binding of [^{3}H]-labelled hexasaccharides derived from *Pneumococcus* type 3 polysaccharide with anti-type 3 antibodies has been studied by equilibrium dialysis.[128] The hexasaccharide with a non-reducing terminal D-glucopyranosyluronic acid residue (35) was bound more tightly than the second hexasaccharide (36) indicating that such terminal units constituted the major portion of the binding energy.

Native and partially de-xylosylated polysaccharides from *Tremella mesenterica* and *Cryptococcus laurenti*, containing D-glucuronic acid units,

[126] T. Miyazaki and J. K. N. Jones, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1531.
[127] M. Heidelberger, N. Roy, and C. P. J. Glaudemans, *Biochemistry*, 1969, **8**, 4822.
[128] M. Katz and A. M. Pappenheimer jun., *J. Immunol.*, 1969, **103**, 491.



(35)



(36)

have been examined with respect to their reaction with horse antipneumococcal type 2 serum.[129] Differences were observed in the relative ability of sodium D-glucuronate and *p*-nitrophenyl β-D-glucopyranoside to inhibit precipitation of anti-type 2 antibodies with the *Cryptococcus laurentii* polysaccharide, oxycellulose, and *Pneumococcus* type 2 polysaccharide. The polysaccharide from *T. mesenterica* did not cross-react with this antiserum.

The immunochemical relationships of certain *Streptococcus* group- and type-specific polysaccharides to the *Pneumococcus* capsular antigens has enabled correlations of structure to be made.[130] A specific F antigen was recognised and one of five type-specific polysaccharides. Some strains also contained some group-like polysaccharide antigens in addition to one of the type-specific polysaccharides. The type 2 polysaccharide from group F *Streptococcus* contained 2-acetamido-2-deoxy-D-galactose, D-galactose, L-rhamnose, and D-glucose (1 : 1 : 2 : 2).[131] Partial acid hydrolysis gave a mixture of four disaccharides, and three higher oligosaccharides including a pentasaccharide containing the four sugars present in the polymer in the mole ratio 1 : 1 : 1 : 2. This pentasaccharide was characterised as (37), and

β-D-Gal*p*NAc-(1 → 2)-*O*-α-D-Gal*p*-(1 → 2)-*O*-α-L-Rha*p*-(1 → 4)-*O*-β-D-G*p*-(1 → 4)-D-G

(37)

shown to be the immunodeterminant feature of the antigen. Terminal β-(2-acetamido-2-deoxy-D-glucopyranosyl) residues were the prominent

[129] C. M. Helms, P. Z. Allen, and D. S. Feingold, *Immunochem.*, 1969, **6**, 269.
[130] M. Heidelberger, J. M. N. Willers, and M. F. Michel, *J. Immunol.*, 1969, **102**, 1119.
[131] M. F. Michel, J. van Vonno, and R. M. Krause, *J. Immunol.*, 1969, **102**, 215.

antigenic determinant of the cell-wall carbohydrate of *Streptococcus bovis* S19.[132] The polysaccharide responsible for group G *Streptococcus* antigenicity has been isolated.[133] On the basis of methylation studies and the inhibition of group G specific precipitin reaction by various rhamnosyl derivatives it was concluded that the serological determinants were terminal L-rhamnose units linked α-(1 → 4) to the penultimate L-rhamnose unit. It was tentatively suggested that the determinants of groups A, C, E, and H activity were terminal α-(1 → 3) linked L-rhamnose units, whilst those for groups G, F, and K were α-(1 → 4) linked and group B α-(1 → 2) linked. The metabolism of the reserve polysaccharide from *S. mitis* has been examined.[134] Iodophilic polysaccharide synthesis, acid production, and growth in oral Streptococci have been studied.[135]

The polysaccharide slimes of *Pseudomonas aeruginosa* have been compared.[136] Essentially similar polysaccharides were obtained from eight strains and contained glucose with smaller amounts of mannose, rhamnose, and 2-amino-2-deoxy-glucose. A lipopolysaccharide has been isolated from the cell walls and acetone-dried whole cells of *P. aeruginosa* NCTC 1999.[137] This polymer contained a similar amino-sugar backbone to the lipid portion of enterobacterial lipopolysaccharides, but contained different hydroxy-acids. Glucose, rhamnose, a heptose, and 3-deoxy-octulosonic acid accounted for less than 20% of the lipopolysaccharide. In addition 2-amino-2-deoxy-galactose, 2-amino-2-deoxy-fucose, and alanine were detected in hydrolysates of the polysaccharide moiety. The characterisation of the lipopolysaccharide–protein complex released from cell walls of *P. aeruginosa* by edta has been reported.[138]

The distribution of 3-deoxy-octulosonic acid and sialic acid in *Neisseria meningitidis* has been studied, and their possible confusion in spectrophotometric analysis methods discussed.[139] Polysaccharides have been isolated and characterised from groups A, B, and C *N. meningitidis*.[140] Immunological investigations showed that these polysaccharides were the group-specific antigens.[141] The group A polysaccharide contained at least 86% of an *O*-acetylated 2-acetamido-2-deoxy-mannose phosphate.[140] The group B polysaccharide contained at least 86% *N*-acetyl-neuraminic acid, whilst that from group C contained a corresponding proportion of

[132] J. A. Kane and W. W. Karakawa, *J. Immunol.*, 1969, **102**, 870.
[133] D. T. Chiongle and J. A. Hayashi, *Arch. Biochem. Biophys.*, 1969, **130**, 39.
[134] G. J. Walker, M. C. Lukas, and A. Lavrova, *Carbohydrate Res.*, 1969, **9**, 387.
[135] J. van Houte, K. C. Winkler, and H. M. Jansen, *Arch. Biochem. Biophys.*, 1969, **14**, 45.
[136] M. R. W. Brown, J. H. Scott Foster, and J. R. Clamp, *Biochem. J.*, 1969, **112**, 521.
[137] A. H. Fenson and G. W. Gray, *Biochem. J.*, 1969, **114**, 185.
[138] S. W. Rogers, H. E. Gilleland jun., and R. G. Eagon, *Canad. J. Microbiol.*, 1969, **15**, 743.
[139] E. Hackenthal, *J. Immunol.*, 1969, **102**, 1099.
[140] E. C. Gotschlich, T. Y. Liu, and M. S. Artenstein, *J. Exp. Med.*, 1969, **129**, 1349.
[141] I. Goldschneider, E. C. Gotschlich, and M. S. Artenstein, *J. Exp. Med.*, 1969, **129**, 1327.

N,O-diacetyl-neuraminic acid. Both the group A and C polysaccharides were non-toxic to mice and guinea-pigs, and were good immunogens in human volunteers.[142]

The cell-wall lipopolysaccharide of *N. catarrhalis* contained D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose and -D-galactose, lipid A, ethanolamine, fatty acids, and phosphate.[143] Unlike the lipopolysaccharide of *N. perflava* it lacked a heptose and 3-deoxy-octulosonic acid core which suggested, with other evidence, that *N. catarrhalis* was a taxonomically 'false neisseria'.

A strain-specific lipopolysaccharide has been isolated from two strains of *Rhizobium trifolii* by hot phenol extraction.[144] The lipopolysaccharide was antigenic and showed features common to those found in the Enterobacteriaceae. The *R. trifolii* lipopolysaccharide was unusual, however, in that it contained D-glucuronic acid and in having a low phosphorus content. The immunochemistry of acetyl and pyruvate substituents found in the polysaccharides of several *Rhizobium* species has been studied.[145] Removal of the pyruvate substituent rendered the polysaccharide inactive towards the homologous antiserum and also towards antipneumococcal type 27 serum (the type-specific polysaccharide of *Pneumococcus* type 27 also contained pyruvate ketals). The pyruvate-free polysaccharide showed serological cross-reactions with antipneumococcal sera not observed with the parent polysaccharide.

The linkage between mycollic acid and the arabinogalactan in phenol-treated cell walls of *Mycobacterium tuberculosis* and *M. smegmatis* has been investigated.[146] It was concluded that mycollic acid was attached to arabinofuranosyl residues in the mycollic acid–arabinogalactan–muco-peptide complex. A study of the polysaccharides from *M. tuberculosis* H37Ra, *M. konsasii*, scotochromogenic and Battey strains, showed that they were arabinogalactans containing identical antigenic determinants.[147] The glycogens from *M. pheli*, *M. smegmatis*, and *M. tuberculosis* at various stages of cellular synthesis and degradation have been characterised.[148]

Acetolysis of the dextran of *Leuconostoc mesenteriodes* B followed by deacetylation gave five trisaccharides.[149] Isomaltotriose (0·12%), 3-*O*-α-isomaltosyl-D-glucose (0·8%), 6-*O*-α-nigerosyl-D-glucose (0·43%), 3,6-di-*O*-α-D-glucosyl-D-glucose (0·18%), and nigerotriose (0·12%) were obtained, and the isolation of the latter suggested that some of the α-(1 → 3)-linked units must be contiguous in the original dextran.

[142] E. C. Gotschlich, I. Goldschneider, and M. S. Artenstein, *J. Exp. Med.*, 1969, **129**, 1367.
[143] G. A. Adams, T. G. Tornabene, and M. Yaguchi, *Canad. J. Microbiol.*, 1969, **15**, 365.
[144] B. Humphrey and J. M. Vincent, *J. Gen. Microbiol.*, 1969, **59**, 411.
[145] W. F. Dudman and M. Heidelberger, *Science*, 1969, **164**, 954.
[146] F. Kanetsuna, T. Imaeda, and G. Cunto, *Biochim. Biophys. Acta*, 1969, **173**, 341.
[147] S. E. Birnbaum and L. F. Affronti, *J. Bacteriol.*, 1969, **100**, 58.
[148] A. D. Antoine and B. S. Tepper, *Arch. Biochem. Biophys.*, 1969, **134**, 207.
[149] F. Yamauchi and K. Matsuda, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 103.

Bifidan, a polysaccharide from *Lactobacillus bifidus*, contained D-glucose, D-galactose, 6-deoxy-L-talose, and D-galacturonic acid.[150] The viscosity of a solution of bifidan was irreversibly decreased by ascorbic acid. The depolymerisation was inhibited by peroxidase or sodium diethyldithiocarbamate, and it was suggested that hydroperoxyl radicals were responsible.

A neutral glucan has been isolated from *Pullularia fermentans* var. *fermentans*.[151] The polysaccharide ($[\alpha]_D$ +162° and DP 250) appeared to contain (1 → 4)- and (1 → 6)-linked units on the basis of periodate oxidation studies. Partial acid hydrolysis gave isomaltose, panose, and higher oligosaccharides but no (1 → 3)-linked fragments. It was concluded that the polymer was an α-(1 → 4), α-(1 → 6)-D-glucan (2 : 1).

Some structural features of an acidic exocellular polysaccharide from *Alcaligenes faecalis* var. *myxogenes* 10C3 have been elucidated.[152] The polysaccharide ($[\alpha]_D$ −15°) contained D-glucose, D-galactose, and succinic acid (7 : 1 : 1·5). The succinic acid was removed by hydrolysis to give a neutral polysaccharide ($[\alpha]_D$ −26°) which was oxidised by periodate at a similar rate to the parent polysaccharide. Methylation and periodate oxidation indicated that the D-galactose residues were 3-substituted, whilst the D-glucose units were 4-, 6-, or 4,6-substituted. Smith degradation gave 2-*O*-β-D-galactopyranosyl-erythritol, 2-*O*-β-D-glucopyranosyl-erythritol, 2-*O*-β-laminaribiosyl-erythritol, 1-*O*-β-laminaribiosyl-glycerol, and small amounts of glycerol and erythritol. On the basis of this evidence, partial structural features (38) of the succinogalactoglucan were recognised.

→ 4)-β-D-G*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-G*p*-(1 →
→ 4)-β-D-G*p*-(1 → 3)-β-D-G*p*-(1 → 4)-β-D-G*p*-(1 →
→ 3)-β-D-G*p*(1 → 3)-β-D-G*p*-(1 → 6)-β-D-G*p*-(1 →
→ 3)-β-D-G*p*(1 → 3)-β-D-G*p*-(1 → 4)-β-D-G*p*-(1 →

β-D-G*p*
1
↓
6
→ 4)-β-D-G*p*-(1 → (38)

A capsular polysaccharide containing L-rhamnose and D-glucose (4 : 1) has been isolated from *Acinetobacter calco-aceticus* (formerly *Bacterium anitratum*).[153] The rhamnoglucan precipitated a number of antistreptococcal group B sera as well as antistreptococcal group G and antipneumococcal type 23 sera. Non-reducing terminal L-rhamnosyl groups were known to be the determinant of the antigens giving rise to these antisera, and

[150] M. M. Wang, K. C. Tsou, and R. F. Norris, *Arch. Biochem. Biophys.*, 1969, **131**, 513.
[151] E. Ninomiya and T. Kizaki, *J. Agric. Chem. Soc. (Japan)*, 1969, **43**, 115.
[152] A. Misaki, H. Saito, T. Ito, and T. Harada, *Biochemistry*, 1969, **8**, 4645.
[153] M. Heidelberger, A. Das, and E. Juni, *Proc. Nat. Acad. Sci.*, 1969, **63**, 47.

non-reducing terminal residues of L-rhamnose were postulated for this polysaccharide. Both the *A. calco-aceticus* rhamnoglucan and *Pneumococcus* type-2-specific polysaccharide precipitated the same portion of anti-pneumococcal type 6 sera, which suggested that a second fraction of the L-rhamnose was present in the form of (1 → 3) linkages. A portion of the L-rhamnose residues were resistant to periodate oxidation.

Immunologically active polysaccharides have been extracted from the cell walls of *Listeria monocytogenes* types 1, 2, 3, 4a and 4b.[154] Types 1 and 2 polysaccharides contained rhamnose as the immunodominant sugar, whilst the galactoglucans of types 4a and 4b contained galactose and glucose as the respective major antigenic determinant.

The mannan formed enzymically from mannosyl-1-phosphoryl-undecaprenol has been characterised.[155] Methylation indicated that (1 → 2)-, (1 → 3)-, and (1 → 6)-linked units were present with a low degree of branching. *In vitro* synthesis with [^{14}C]-labelled precursor resulted in the formation of a mannan with [^{14}C]-D-mannopyranosyl terminal non-reducing residues. *In vivo* experiments resulted in a uniformly labelled mannan. It was considered that *de novo* synthesis had not been achieved with the majority of mannosyl residues incorporated as single non-reducing termini with a small proportion of double incorporation.

The thermophilic bacteria *Bacillus stearothermophilus* has been shown to contain an ADP-glucose, glucan-1,4-glucosyltransferase, which synthesised a polysaccharide non-identical with glycogen.[156]

The comparative composition of the cell-wall lipopolysaccharide and extracellular polysaccharide from *Brucella melitensis* has been reported.[157] The major difference appeared to be the presence of lipid A in the lipopolysaccharide. Both polymers contained 3-deoxy-octulosonic acid, D- and L-*glycero*-D-*manno*-heptose, D-glucose, D-galactose, and colitose or abequose and a small proportion of rhamnose in the polysaccharide and arabinose in the lipopolysaccharide (possibly contaminants).

Further investigations have been reported on the antigenic structure of the polysaccharides from S forms [158, 159] and R forms [160] of *Proteus mirabilis*.

A new method has been described for the extraction of lipopolysaccharides from R forms of bacteria.[161] The R-form polymer was soluble in a monophasic extraction mixture of aqueous phenol–chloroform–petroleum ether, whilst S- and T-form lipopolysaccharides were insoluble.

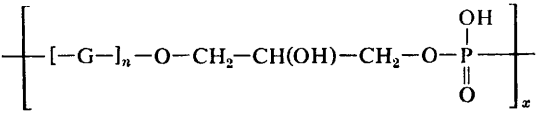
[154] W. W. Ullmann and J. A. Cameron, *J. Bacteriol.*, 1969, **98**, 486.
[155] M. Scher and W. J. Lennarz, *J. Biol. Chem.*, 1969, **244**, 2777.
[156] S. H. Goldemberg and I. D. Algranati, *Biochim. Biophys. Acta*, 1969, **177**, 166.
[157] C. Lacave, J. Asselineau, A. Serre, and J. Roux, *European J. Biochem.*, 1969, **9**, 189.
[158] K. Kotelko, W. Gromska, Z. Sidorczyk, and J. Zwolinski, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1968, **16**, 739.
[159] K. Kotelko, J. Radziejewska, Z. Sidorczyk, K. Izdebska-Szymona, and J. Zwolinski, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1968, **16**, 745.
[160] W. Gromska, J. Radziejewska, Z. Sidorczyk, and J. Zwolinski, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1969, **17**, 19.
[161] C. Galanos, O. Luderitz, and O. Westphal, *European J. Biochem.*, 1969, **9**, 245.

An immunochemical study of the polysaccharides of the complete antigens of the alkali-forming Enterobacteria has been reported.[162] The physicochemical properties of a lipopolysaccharide from *Streptoverticillium* species 0223[163] and excreted lipopolysaccharide–phospholipid–protein complex have been studied.[164]

The immunochemistry of a formamide-extracted antigen from *Clostridium perfringens* cell-wall polysaccharide or mucopeptide has been described.[165]

Bacterial Cell-wall Materials

A teichoic acid from cell walls of *Lactobacillus plantarum* lacking the group D precipitinogen, a glucosyl-ribitol teichoic acid, has been investigated.[166] These strains contained a glucosyl-glycerol teichoic acid, in which the glucosyl substituents were attached to a primary hydroxy-group of glycerol. Three distinct repeating units were isolated from the teichoic acid preparation from strain C106, indicating either a complex polymer or a mixture of teichoic acids. The absence of glycerol diphosphates and the presence of glucose phosphate in acid hydrolysates indicated that the phosphodiester connected the glucose and glycerol functions. Enzymic dephosphorylation of the mixture of glucosyl-glycerolphosphates from the acid hydrolysate gave a mixture of two isomeric diglucosylglycerols and a monoglucosyl-glycerol. A representative structure (39) was suggested in



$n = 1$ or 2 (39)

which the location of the alanyl residues was not included. The distribution of the glucosyl substituents along the chains of teichoic acids from walls of *L. buchneri* NCIB 8007 has been established.[167] Since alkaline degradation to glucosyl-glycerol only occurred when glucosyl-glycerol residues in the teichoic acid were flanked by unsubstituted glycerol phosphate residues, this allowed the determination of the glucosyl distribution. This also permitted distinction of an integral polymer from a mixture of polyglycerol phosphate and fully glucosylated polyglycerol phosphate. This teichoic acid (glucose : phosphate, 1 : 4) gave glucosyl-glycerol on alkaline degradation equivalent to 50% of the total hexose content. This was the theoretical

[162] G. M. Laushnik and E. Ya. Rashba, *Z. Mikrobiol. Epidemiol. Immunobiol.*, 1969, 12.
[163] T. P. Efumoia, O. N. Ekzemplyarov, and V. A. Tsyganov, *Antibiotiki*, 1969, **14**, 119.
[164] L. Rothfield and M. Pearlman-Kothencz, *J. Mol. Biol.*, 1969, **44**, 477.
[165] H. M. Johnson, K. Brenner, and H. E. Hall, *J. Bacteriol.*, 1969, **100**, 176.
[166] J. B. Adams, A. R. Archibald, J. Baddiley, H. E. Coapes, and A. L. Davison, *Biochem. J.*, 1969, **113**, 191.
[167] A. R. Archibald, J. Baddiley, and S. Heptinstall, *Biochem. J.*, 1969, **111**, 245.

yield from a randomly substituted polymer of this composition. The formation of glucosyl-glycerol did not depend on prior acid treatment.

The extraction of bacterial cell walls by dilute alkali has been suggested as a valuable alternative to previous extraction procedures for teichoic acids.[168] Teichoic acids isolated by this procedure were still polymeric and even in cases where extensive dissolution of the mucopeptide occurred it was obtained from mucopeptide components. Although the procedure removed alanine ester substituents, it avoided acid hydrolysis. Teichoic acids were solubilised and isolable in high yields after treatment of bacterial cell walls with dilute aqueous solutions of *NN*-dimethylhydrazine in the presence of air.[169] This procedure was suggested as a valuable technique for the examination of the distribution of heteropolymers in cell walls and was used to show the absence of typical teichoic acids in *L. arabinosus* cell walls.

The control of teichoic acid and teichuronic acid biosynthesis in chemostat cultures of *Bacillus subtilis* has been studied.[170, 171] The structure of a teichoic acid from the cell walls of *Actinomyces violaceus* has been studied.[172]

A number of reports have been concerned with the structural and biosynthetic aspects of the peptidoglycans of bacterial cell walls. The peptidoglycan of *Lactobacillus casei* R094 consisted of glycan chains of alternating β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranosyl and *N*-acetyl-muramic acid residues with an average chain length of ten disaccharide units.[173] Each *N*-acetyl-muramic acid residue was substituted with the pentapeptide N^{α}-(L-ala-D-isoglutaminyl)-N^{ε}-(D-isoasparginyl)-L-lys-D-alanine. Cross-linkages were *via* random (55%) D-alanine to D-isoasparagine. A similar peptidoglycan was isolated from the vegetative cell walls of *Bacillus sphaericus* 9602.[174] The sole difference observed was the lack of the *C*-terminal D-alanine residues and the amidation of the glutamic acid α-carboxy-group. The cell-wall peptidoglycans of *Staphylococcus aureus* Copenhagen and *S. epidermidis* Texas 26 both have strict alternation of 2-acetamido-2-D-glucose and *N*-acetyl-muramic acid residues in the glycan portion.[175] These materials also contained a teichoic acid substituted by D-alanyl and 2-acetamido-2-deoxy-D-glucosyl residues. The structures of the neutral and basic peptide constituents were established by enzyme degradation.[176]

[168] A. R. Archibald, H. E. Coapes, and G. H. Stafford, *Biochem. J.*, 1969, **113**, 899.
[169] J. C. Anderson, A. R. Archibald, J. Baddiley, M. J. Curtis, and N. B. Davey, *Biochem. J.*, 1969, **113**, 183.
[170] D. C. Ellwood and D. W. Tempest, *Biochem. J.*, 1969, **111**, 1.
[171] D. C. Ellwood, W. H. Turner, J. R. Hunter, and G. R. G. Moody, *Biochem. J.*, 1969, **113**, 14P.
[172] I. B. Naumova, S. V. Rogozina, and N. M. Mirsalikhova, *Doklady Akad. Nauk. S.S.S.R.*, 1969, **188**, 710.
[173] K. D. Hungere, J. Fleck, and D. J. Tipper, *Biochemistry*, 1969, **8**, 3567.
[174] K. D. Hungere and D. J. Tipper, *Biochemistry*, 1969, **8**, 3577.
[175] D. J. Tipper and M. F. Berman, *Biochemistry*, 1969, **8**, 2183.
[176] D. J. Tipper, *Biochemistry*, 1969, **8**, 2192.

The mechanism of biosynthesis and direction of chain extension of a poly-(2-acetamido-2-deoxy-D-glucose-1-phosphate) from walls of *S. lactis* NCTC 2102 have been investigated.[177] Pulse-labelling showed that chain extension was *via* transfer from the nucleotide to the end of the chain that was *not* attached to the peptidoglycan in the wall. The C-6-hydroxy-group of the terminal 2-acetamido-2-deoxy-D-glucose residue in the polymer was indicated as the acceptor site.[178]

The mechanism of autolysis of isolated cell walls of *S. aureus* has been investigated.[179]

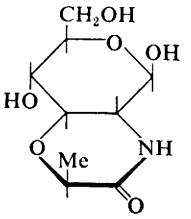
The chemical composition of the cell envelope of *Streptobacillus moniliformis* was studied to look for features to account for the ability of this organism to form L forms spontaneously.[180] The low levels of 2-acetamido-2-deoxy-D-glucose and *N*-acetyl-muramic acid determined suggested that an unusually low mucopeptide content was associated with the tendency to give L forms.

N-Glycollyl-muramic acid has been identified in the cell wall extracts of *Mycobacterium smegmatis* with the aid of mass spectrometry.[181] Di- and tetra-saccharides were isolated from the walls by enzyme degradation which differed from those obtained from *M. lysodeikticus* presumably due to the presence of *N*-glycollyl-muramic acid.[182] A chemical analysis of the mucopeptide from *M. smegmatis* has been reported,[183] but not the nature of the acylation of the muramic acid residues. This arabinogalactan–mucopeptide complex appeared to have a similar composition to other diaminopimelic acid-containing bacterial mucopeptides in other respects.

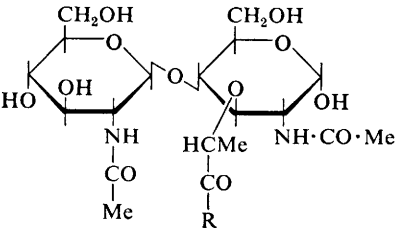
Six major oligosaccharides were isolated from the peptidoglycan of spores from *Bacillus subtilis* by lysozyme treatment.[184] These consisted of di-, tetra-, and hexa-saccharides of alternate muramic acid and 2-amino-2-deoxy-D-glucose residues (with 2, 3, and 4 acetyl groups respectively) substituted by either a single L-alanyl residue, or a single tetrapeptide on *N*-acetyl-muramic acid at the reducing ends of the oligosaccharides. The other muramic acid residues in the tetra- and hexa-saccharides were present as a muramic acid lactam (40). The facile reduction of this residue by sodium borohydride-[^{3}H] provided a sensitive detection system for this compound. Although 55% of all the muramic acid present in the spores was in the form of the lactam, none was detected in the cell walls of the organism. The glycosidic bond to the muramic acid lactam was resistant to cleavage by lysozyme. Regular spacing of the lactam units along the

[177] D. Brooks and J. Baddiley, *Biochem. J.*, 1969, **113**, 635.
[178] D. Brooks and J. Baddiley, *Biochem. J.*, 1969, **115**, 307.
[179] D. J. Tipper, *J. Bacteriol.*, 1969, **97**, 837.
[180] L. H. Knipp and J. R. Sokatsch, *Canad. J. Microbiol.*, 1969, **15**, 665.
[181] A. Adam, J. F. Petit, J. Wietzerbin-Falszpan, P. Sinaÿ, D. W. Thomas, and E. Lederer, *F.E.B.S. Letters*, 1969, **4**, 87.
[182] J. F. Petit, A. Adam, J. Wietzerbin-Falszpan, E. Lederer, and J. M. Ghuysen, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 478.
[183] G. Cunto, F. Kanetsuna, and T. Imaeda, *Biochim. Biophys. Acta*, 1969, **192**, 358.
[184] A. D. Warth and J. L. Strominger, *Proc. Nat. Acad. Sci.*, 1969, **64**, 528.

chains was evident since three times as much tetrasaccharide (42) as disaccharide (41) was formed.



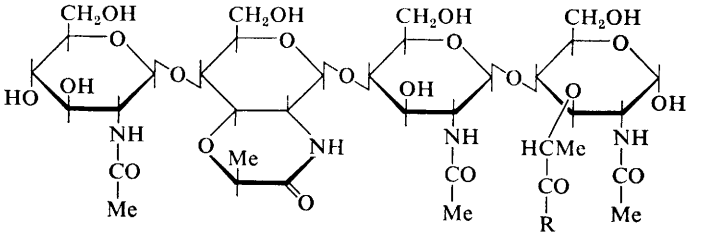
(40)



R = L-Ala

or L-Ala-D-γ-Glu-*meso*-DAP-D-Ala

(41)



(42)

An improved technique has been described for the preparation of a *Streptomyces* *N*-acetyl-muramyl-L-alanine amidase.[185] This enzyme was employed to obtain a disaccharide from the peptidoglycan of *Butyribacterium rettgeri* after initial degradation with an *endo*-*N*-acetylmuramidase.[186] This confirmed that the glycan was composed entirely of β-4-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-*N*-acetyl-muramic acid. The peptide fragments were composed of N^{α}-(L-seryl-γ-D-glutamyl)-L-ornithyl-D-alanine subunits, cross-linked by D-lysine or D-ornithine (2 : 1) to the α-carboxy-group of glutamic acid and to the alanine on the next unit. A similar glycan fraction was found in the cell wall of *M. lysodeikticus*, *M. flavus*, *M. citreus*, and *Sarcina lutea* although not all of the *N*-acetylmuramic acid residues were substituted.[187] The peptide subunits of these peptidoglycans were composed of N^{α}-[L-alanyl-γ-(α-D-glutamylglycine)]-L-lysyl-D-alanine cross-linked through D-alanyl-L-alanyl and N^{ε}-(D-alanyl)-L-lysyl bridges with no intervening amino-acids. In *M. lysodeikticus*, *M. flavus*, and *S. lutea* segments of the glycan of 8 to 18 disaccharide units

[185] J. M. Ghuysen, L. Dierickx, J. Coyette, M. Leyh-Bouille, M. Guinand, and J. N. Cambell, *Biochemistry*, 1969, **8**, 182.

[186] M. Guinand, J. M. Ghuysen, K. H. Schleifer, and O. Kandler, *Biochemistry*, 1969, **8**, 200.

[187] J. N. Cambell, M. Leyh-Bouille, and J. M. Ghuysen, *Biochemistry*, 1969, **8**, 193.

were linked through a *N*-acetyl-muramic acid 1-phosphate to a segment of a second polymer. β-4-*O*-(2-Acetamido-2-deoxy-D-glucopyranosyl)-*N*-acetyl-muramyl-L-alanyl-γ-D-glutamyl-(L)-*meso*-diaminopimelic acid-(L)-D-alanine was isolated from the cell envelope of *E. coli* B.[188]

Alkaline degradation of the phosphomannanpeptide from yeast cell walls gave α-aminobutyric acid and alanine residues from serine and threonine, and also released mannose-containing oligosaccharides.[189] The kinetics and solubilisation of phospho-*N*-acetyl-muramyl pentapeptide translocase (UDP-5′-phosphate) have been studied.[190]

Further studies have been reported on the specificity and mode of action of lysozyme.[191–212]

Fungal and Yeast Polysaccharides

Heterogalactans from several species of *Polyporus* have been studied. The polysaccharides elaborated by *Polyporus fomentarius* (Fr.) and *P. igniarius* (Fr.) were structurally similar, and contained a mannofucogalactan and a glucuronoglucan.[213] The mannofucogalactan consisted of an α-(1 → 6)-D-galactopyranosyl backbone with 30—40% substitution by either L-fucopyranose, 3-*O*-α-D-mannopyranosyl-L-fucopyranose, or D-galactopyranose units (43). A somewhat similar polysaccharide was elaborated by *P.*

[188] J. van Heijenoort, L. Elbaz, P. Dezelee, J. F. Petit, E. Bricas, and J. M. Ghuysen, *Biochemistry*, 1969, **8**, 207.
[189] T. N. Cawley and R. Letters, *Biochem. J.*, 1969, **115**, 9P.
[190] M. G. Heydanek jun., W. G. Struve, and F. C. Neuhaus, *Biochemistry*, 1969, **8**, 1214: 1247.
[191] D. Piszkiewicz and T. C. Bruice, *Arch. Biochem. Biophys.*, 1969, **129**, 317.
[192] J. J. Pollock and N. Sharon, *Biochem. Biophys. Res. Comm.*, 1969, **34**, 673.
[193] K. Hayashi, N. Fujimoto, M. Kugimiya, and M. Funatsu, *J. Biochem.*, 1969, **65**, 401.
[194] K. Ikeda and K. Hamaguchi, *J. Biochem.*, 1969, **66**, 513.
[195] T. Imoto, Y. Doi, K. Hayashi, and M. Funatsu, *J. Biochem.*, 1969, **65**, 667.
[196] G. L. Rossi, E. Holler, S. Kumar, J. A. Rupley, and G. P. Hess, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 757.
[197] R. M. Parry jun., R. L. Chandan, and K. M. Shahani, *Arch. Biochem. Biophys.*, 1969, **130**, 59.
[198] F. W. Dahlqvist, C. L. Borders jun., G. Jacobson, and M. A. Raftery, *Biochemistry*, 1969, **8**, 694.
[199] S. M. Parsons, Z. Jao, F. W. Dahlqvist, C. L. Berders jun., T. Groff, J. Racs, and M. A. Raftery, *Biochemistry*, 1969, **8**, 700.
[200] L. W. Black and D. S. Hogness, *J. Biol. Chem.*, 1969, **244**, 1982.
[201] B. D. Sykes, *Biochemistry*, 1969, **8**, 1110.
[202] M. Z. Attasi and A. F. S. A. Habeeb, *Biochemistry*, 1969, **8**, 1385.
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[204] T. Rand-Meir, F. W. Dahlqvist, and M. A. Raftery, *Biochemistry*, 1969, **8**, 4206.
[205] F. W. Dahlqvist, T. Rand-Meir, and M. A. Raftery, *Biochemistry*, 1969, **8**, 4214.
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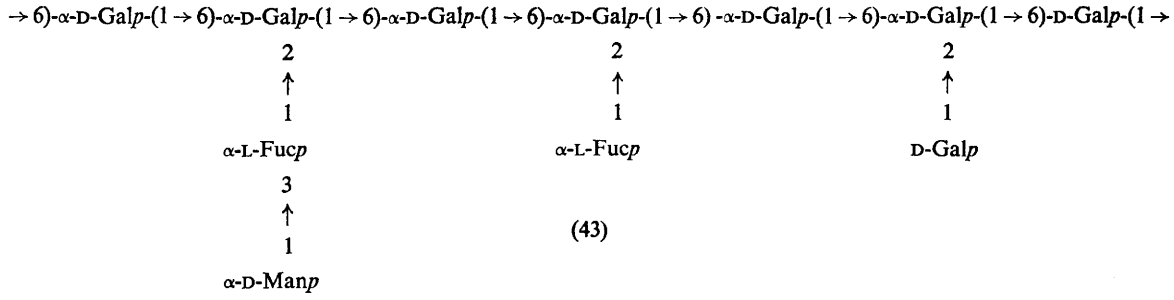
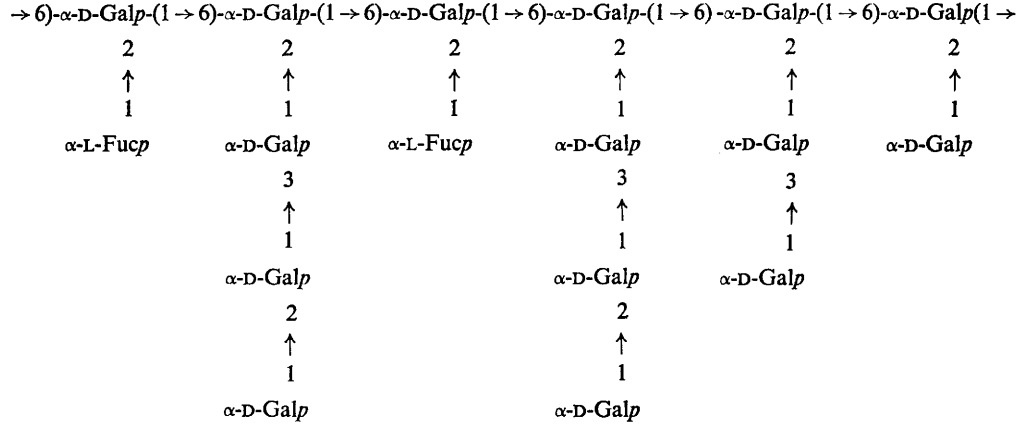
[207] K. C. Aune and C. Tanford, *Biochemistry*, 1969, **8**, 4586.
[208] T. Y. Liu and D. E. Koshland jun., *J. Biol. Chem.*, 1969, **244**, 505.
[209] L. W. Black and D. S. Hogness, *J. Biol. Chem.*, 1969, **244**, 1968; 1976.
[210] A. J. Sophianopoulos, *J. Biol. Chem.*, 1969, **244**, 3188.
[211] A. N. Glazer, A. O. Barel, J. B. Horard, and D. M. Brown, *J. Biol. Chem.*, 1969, **244**, 3583.
[212] C. J. Kowalski and P. R. Schimmel, *J. Biol. Chem.*, 1969, **244**, 3643.
[213] H. Bjorndal and B. Lindberg, *Carbohydrate Res.*, 1969, **10**, 79.


→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-D-Galp-(1→
2
1
α-L-Fucp
3
1
α-D-Manp
2
1
α-L-Fucp
2
1
D-Galp

(43)


→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp(1→
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α-D-Galp
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α-D-Galp
2
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α-D-Galp
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α-L-Fucp
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1
α-D-Galp
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1
α-D-Galp
3
1
α-D-Galp
2
1
α-D-Galp

(44)

squamosus.[34] α- and β-Glucans were removed from a water-soluble extract by precipitation with boric acid–cetyltrimethylammonium hydroxide to give a polysaccharide that contained D-galactose and L-fucose (7·5 : 1), together with small quantities of D-mannose (2—3 mol %). Methylation before and after mild hydrolysis indicated that the backbone of the polymer was composed of D-galactopyranose units mutually joined by α-(1 → 6) linkages with *ca.* 90% C-2 substitution by either α-L-fucopyranosyl, 3-*O*-α-D-mannopyranosyl-α-L-fucopyranosyl or short chains of α-(1 → 2)- and α-(1 → 3)-linked D-galactopyranose units. Methylation of the polysaccharide after selective cleavage at hexose units unsubstituted at the C-6 position indicated that the L-fucopyranosyl residues were linked directly to the α-(1 → 6)-D-galactan backbone. A probable repeating sequence (44) was suggested for this polysaccharide.

The water-soluble neutral polysaccharides from the fruit bodies of *Polyporus borealis* have been fractionated to give a mannan ($[\alpha]_D$ +82°; molecular weight 350,000) and a fucogalactan ($[\alpha]_D$ +92°; molecular weight 179,000).[214] On the basis of methylation studies, a hypothetical structure (45) was proposed for the mannan. Methylation before and after removal of the L-fucose residues indicated that the fucogalactan was an α-(1 → 6)-D-galactan with 35% substitution by L-fucopyranosyl units at the C-2 position (46). The fucogalactan from *P. ovinus* ($[\alpha]_D$ +85°) contained D-galactose and L-fucose (3·6 : 1).[215] Structural analysis revealed that this polysaccharide was entirely analogous to the fucogalactan from *P. borealis* with a lower L-fucose content. *Polyporus pinacola* (Fr.) gave, in contrast, a fucoxylomannan ($[\alpha]_D$ −42°).[216] This polysaccharide contained L-fucose, D-xylose, and D-mannose (0·8 : 0·7 : 1·0), with traces of D-glucose and D-galactose. Methylation analysis indicated a β-(1 → 3)-D-mannan backbone substituted at the C-4 positions by β-(2-*O*-α-L-fucopyranosyl)-D-xylopyranosyl residues (46a).

A hot-water extract of *Lentinus edodes* gave four polysaccharides after fractionation.[217] Two of these fractions, LC33 and LC1, exhibited antitumour activity (against mouse sarcoma 180). LC33 Polysaccharide ($[\alpha]_D$ +19·5—21·5°), from the results of acetolysis and enzymic degradation, was a linear β-(1 → 3)-D-glucan. However, the physical and biological properties were different from those of the β-(1 → 3)-D-glucan, β-pachyman, from *Poria cocos*. LC1 Polysaccharide gave cellobiose and gentiobiose on partial hydrolysis and on acetolysis, and was apparently a β-(1 → 4)-, β-(1 → 6)-D-glucan. Polysaccharides from *Gyrophora esculenta* Miyoshi, and *Lasallia papulosa* Llamo also showed antitumour activity.[218]

[214] H. Bjorndal and B. Wagstrom, *Acta Chem. Scand.*, 1969, **23**, 1560.
[215] K. Axelsson and H. Bjorndal, *Acta Chem. Scand.*, 1969, **23**, 1815.
[216] K. Axelsson, H. Bjorndal, and B. Lindberg, *Acta Chem. Scand.*, 1969, **23**, 1597.
[217] G. Chihara, Y. Maeda, J. Hamura, T. Sasabi, and F. Fukuoka, *Nature*, 1969, **222**, 687.
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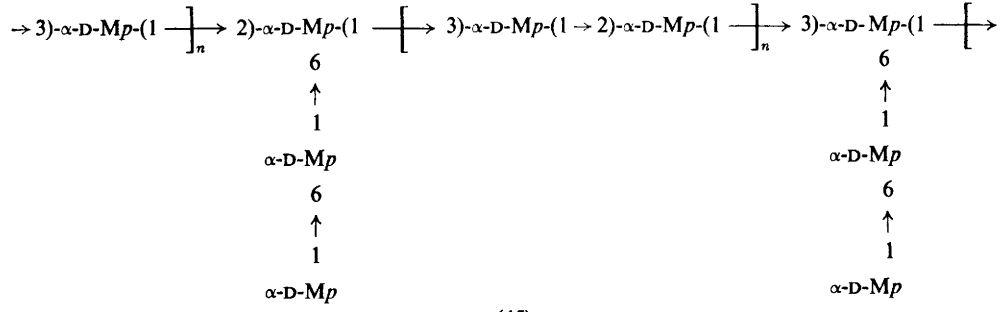
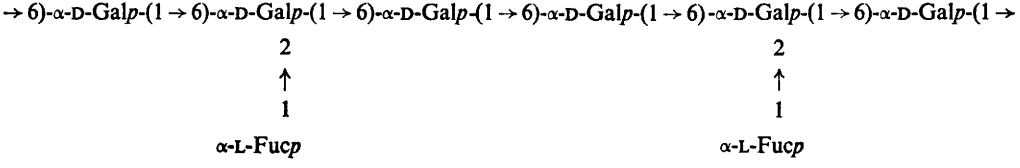
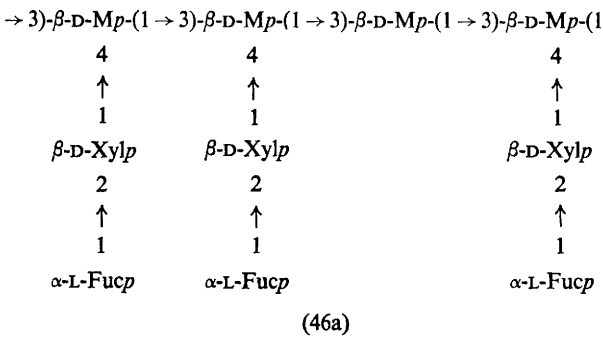

→3)-α-D-Mp-(1
n
2)-α-D-Mp-(1
6
↑
1
α-D-Mp
6
↑
1
α-D-Mp
3)-α-D-Mp-(1→2)-α-D-Mp-(1
n
3)-α-D-Mp-(1
6
↑
1
α-D-Mp
6
↑
1
α-D-Mp

(45)


→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→
2
↑
1
α-L-Fucp
2
↑
1
α-L-Fucp

(46)



(46a)

A glucan ($[\alpha]_D$ +253°) and chitin have been isolated from the yeast form of *Paracoccidiodes brasiliensis*.[219] The chemical composition of the cell-wall material of the yeast form and the mycelial forms of *P. brasiliensis* and *Blastomyces dermatidis* were similar.[220] In addition to the alkali-soluble glucan present in both forms, the mycelial form contained an alkali-insoluble glucan that was hydrolysable by snail digestive juice. The cell wall of the dinoflagellate *Peridinium westii* contained a D-glucan closely related to cellulose, although with different physical properties.[221] Partial hydrolysis or acetolysis gave a series of cellodextrins, $n = 2$—4, together with some laminaritriose and laminaribiose.

The insoluble cell-wall glucan of *Phytopthora cinnamomi* appeared to be a highly branched β-(1 → 3)-D-glucan with about four chain residues per branch point.[222] Methylation indicated branching through C-3 and C-6. Erythritol was obtained amongst the products from a Smith degradation, which suggested the presence of some 4-*O*-substitution.

Pulse-labelling experiments with D-glucose-[^{14}C] has been utilised to examine the biosynthesis of the β-D-glucan of *Sclerotium rolfsii* Sacc.[223] Determination of the specific activity of each type of linkage in the *in vitro* synthesised polysaccharide showed that chain elongation proceeded towards the non-reducing terminal unit. Furthermore, the interbranch, branch point, and branch D-glucose residues were inserted at about the same time. The D-glucose was incorporated without rearrangement.

The n.m.r. spectra of mannans from *Candida* have been suggested as a means of classification.[224] Oligosaccharides have been isolated from the mannan of *Candida albicans* with DP 5—8 containing common features of

[219] R. E. Moreno, F. Kanetsuna, and L. M. Carbonell, *Arch. Biochem. Biophys.*, 1969, **130**, 212.
[220] F. Kanetsuna, L. M. Carbonell, R. E. Moreno, and J. Rodriguez, *J. Bacteriol.*, 1969, **97**, 1036.
[221] Z. Nero and N. Sharon, *Biochim. Biophys. Acta*, 1969, **173**, 161.
[222] L. P. T. M. Zevenhuizen and S. Bartnicki-Garcia, *Biochemistry*, 1969, **8**, 1496.
[223] K. K. Batra, J. H. Nordin, and S. Kirkwood, *Carbohydrate Res.*, 1969, **9**, 221.
[224] J. F. T. Spencer and P. A. J. Gorin, *Antonie van Leeuwenhoek J. Microbiol. Serol.* 1969, **35**, 33.

6-*O*- and 2,6-di-*O*-substituted mannopyranose units.[225] These oligosaccharides were antigenic inhibitors which indicated that the branching parts of the mannan were the antigenic determinant groups. Unbranched α-(1 → 6) oligo-D-mannans isolated by acetolysis were poor inhibitors[226] and therefore consecutive α-(1 → 6)-linked mannopyranose units were not responsible for antigenic specificity. The soluble polysaccharides from *Candida tropicalis* and *C. viswanathii* have been partially characterised.[227]

The carbohydrate compositions of the conidial and hyphal walls of *Penicillium chrysogenum* have been determined.[228] Both conidia and hyphae contained glucose, galactose, and 2-acetamido-2-deoxy-glucose, although in different proportions, with lesser amounts of rhamnose and mannose. Solubilisation of cell walls with chitinase followed by β-(1 → 3)-D-glucanase gave values for the hexose content of the carbohydrate portion of glucose (40%), galactose (4%), and mannose (8%).[229] Laminaritriose was obtained from the chitinase digest and laminaribiose from the subsequent β-(1 → 3)-D-glucanase digest.

An intracellular polysaccharide has been isolated from *P. chrysogenum*.[230] Graded and partial acid hydrolysis and methylation indicated an essentially linear structure containing (*a*) acid-labile 1 → 5-linked D-galactofuranose units, (*b*) smaller amounts of 1 → 3-linked D-galactopyranose units, (*c*) *O*-β-D-mannopyranose-(1 → 3)-D-mannose, and (*d*) *O*-D-mannopyranose-(1 → 2/3)-D-mannopyranose-(1 → 3/2)-D-mannose sections. Some comparative analytical data have been reported for galactomannans isolated from the mycelia and culture filtrates of *P. chrysogenum* and *Aspergillus niger*.[231]

A comparative survey has been made of the relative susceptibility of the cell walls of several *Penicillium* species and other organisms by chitinase and β-(1 → 3), β-(1 → 6)-D-glucanases.[232] The high-molecular-weight fraction of the exocellular polysaccharide of *P. charlesii* G. Smith contained phosphorus that was not released by alkaline phosphatase or phosphodiesterase action.[233] Mild acid hydrolysis released orthophosphate and probably hexose phosphate. Fractionation revealed that the high-molecular-weight fraction was continuously heterogeneous with respect to size and hexose composition (glucose, galactose, and mannose).[234]

The polysaccharides from *Microsporum praecox*, *Trychophyton ferrugenium*, *T. sabouraudii*, and *T. tonsurans* have been separated into two

[225] S. Suzuki and H. Sumayama, *Jap. J. Microbiol.*, 1969, **13**, 95.
[226] S. Suzuki and H. Sumayama, *Jap. J. Microbiol.*, 1969, **12**, 413.
[227] N. P. Elinov and M. D. Surinova, *Biokhimiya*, 1968, **33**, 1272.
[228] V. Rizza and J. M. Kornfeld, *J. Gen. Microbiol.*, 1969, **58**, 307.
[229] F. A. Troy and H. Koffler, *J. Biol. Chem.*, 1969, **244**, 5563.
[230] T. Miyazaki and T. Yadomae, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 361.
[231] O. Sakaguchi, K. Yokota, and M. Suzuki, *Jap. J. Microbiol.*, 1969, **13**, 1.
[232] R. M. Peugra, M. A. Cole, and M. Alexander, *J. Bacteriol.*, 1969, **97**, 1056,
[233] J. F. Preston tert., E. Lapis, S. Westerhouse, and J. E. Gander, *Arch. Biochem. Biophys*, 1969, **134**, 316.
[234] J. F. Preston tert., E. Lapis, and J. E. Gander, *Arch. Biochem. Biophys.*, 1969, **134**, 324.

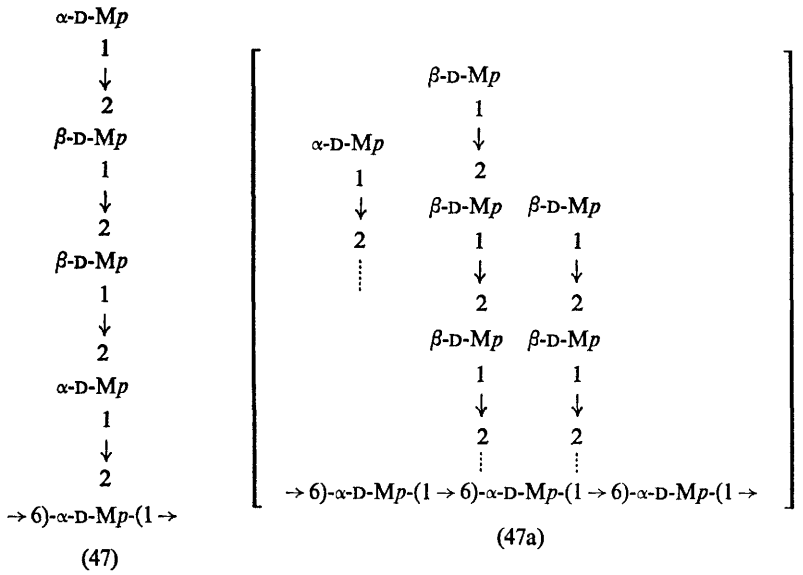
galactomannans and a glucan from each species.[235] Galactomannans 1 from each species were similar, whereas galactomannans 2 were different chemically and in their serological activity. The glucans were also different in structural and serological properties.

N.m.r. spectra have been used as an aid to the classification of yeast mannans and galactomannans by structure.[41, 236] This in turn was used as an aid to the classification of the genera *Hansenula* and *Pichia*.[236] A further method has been suggested for the 'fingerprinting' of yeast mannans based on the products of acetolysis.[237] Controlled acetolysis gave mixtures of oligosaccharides containing predominantly (1 → 2) and (1 → 3) linkages. The distribution patterns obtained by gel permeation chromatography of the mixtures after deacetylation provided a useful basis for taxonomic studies. Use of the *exo*-α-D-mannosidase of *Arthrobacter* GJM-1 for the enzymic degradation of yeast mannans resulted in the formation of polymeric fragments which contained α-(1 → 6) linkages.[238] The enzyme also degraded galactomannans after preferential removal of the D-galactopyranosyl termini by controlled acid hydrolysis. The enzyme appeared to require D-mannopyranosyl non-reducing ends and two consecutive α-D-mannopyranose units for cleavage to occur. Although the enzyme degraded an α-(1 → 2)-D-mannotetraose the tetrasaccharide α-D-M*p*-(1 → 2)-β-D-M*p*-(1 → 2)-β-D-M*p*-(1 → 2)-α-D-M*p*(1 → 2)-D-M*p* was not degraded.

The structures of yeast mannans containing both α- and β-linked units have been determined.[239] Partial hydrolysis in conjunction with n.m.r. and optical rotation measurements indicated that most of the α-linked units were present in the main chains whilst the β-linked units were in the side-chains. Detailed study of the *Pichia pastoris* mannan suggested an α-(1 → 6)-D-mannan main chain substituted at the C-2 positions by a substantial proportion of tetrameric side-chains (47). In contrast the mannan from *Citeromyces matritensis* had a similar main chain but was substituted at the C-2 positions by α-D-mannopyranosyl or β-(1 → 2)-linked D-mannotriosyl residues (47a).

The molecular weights of mannans obtained from yeast cell walls from species NCYC 74 and NCYC 1111 by the action of snail gut juice enzymes were 330,000 and 189,000 respectively.[240] The mannans recovered from alkaline extraction of the cell walls had molecular weights of 121,000 and 85,000. The extraction of the glucan components of the cell wall of *Saccharomyces cerevisiae* has been considered in relation to the ultrastructure of the cell wall.[241] The structures of two glucans from yeast cell

[235] S. F. Grappel, F. Blank, and C. T. Bishop, *J. Bacteriol.*, 1969, **97**, 23.
[236] J. F. T. Spencer and P. A. J. Gorin, *Canad. J. Microbiol.*, 1969, **15**, 375.
[237] J. Kocourek and C. E. Ballou, *J. Bacteriol.*, 1969, **100**, 1175.
[238] P. A. J. Gorin, J. F. T. Spencer, and D. E. Eveleigh, *Carbohydrate Res.*, 1969, **11**, 387.
[239] P. A. J. Gorin, J. F. T. Spencer, and S. S. Bhattacharjee, *Canad. J. Chem.*, 1969, **47**, 1499.
[240] A. A. Eddy and J. Longton, *J. Inst. Brewing*, 1969, **75**, 7.
[241] J. S. D. Bacon, V. C. Farmer, D. Jones, and I. F. Taylor, *Biochem. J.*, 1969, **114**, 557.



(47) (47a)

walls have been investigated.[242] The cell walls of *S. fragilis* contained a high proportion of glucose and mannose (1 : 1).[243]

The cell walls of the air-borne mould *Helminthosporium sativum* contained two distinct hexosamine-containing polymers.[244]

The serological activity of a phenol–water extract of the amoebae *D. discoideum* was traced to a polysaccharide moiety containing 2-acetamido-2-deoxy-glucose, fucose, and mannose (12 : 4·1 : 1).[245]

[242] D. J. Manners and A. J. Mason, *F.E.B.S. Letters*, 1969, **4**, 122.
[243] T. Reuvers, E. Tacoronte, C. G. Mendoza, and M. Novaes-Ledieu, *Canad. J. Microbiol.*, 1969, **15**, 989.
[244] D. A. Applegarth and G. Bozoian, *Arch. Biochem. Biophys.*, 1969, **134**, 285.
[245] G. Gerisch, D. Malchow, H. Wilhelms, and O. Luderitz, *European J. Biochem.*, 1969, **9**, 229.

5
Glycoproteins and Glycopeptides

Interest has continued in the cleavage of the carbohydrate–peptide bonds in glycopeptides by chemical and enzymic means. The determination of hexosaminitols by ion-exchange chromatography has been described and its application to the investigation of alkali-labile linkages in glycoproteins discussed.[246] The purification and characterisation of a 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine amidohydrolase from hen oviduct has been described.[247] This enzyme acted on 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine, ovalbumin, ribonuclease B, and transferrin. A similar enzyme has been isolated from rat liver.[248] Since the purified enzyme preparation was devoid of glycosidase activity, it was considered of value for structural studies. Further studies on the *O*-seryl-*N*-acetyl-galactosaminide glucohydrolase from *Lumbricus terrestris* have characterised the enzyme as an α-*N*-acetyl-D-galactosaminidase which did not act on the corresponding glucoaminide.[249]

The general features of glycoprotein biosynthesis have been discussed.[250] Since the stepwise addition of sugars to the growing chain was directed by specific transferases rather than a template mechanism, the observed heterogeneity of the carbohydrate chains of many glycoproteins was considered to be of biosynthetic origin.

Blood-group Substances.—The method for isolation of blood-group substance A+H from pig stomach linings has been modified to allow the isolation of a biopolymer of higher purity.[251]

An approach to the study of blood-group substance structure, which involves chemical and enzymic degradations, has been proposed.[252] Smith degradation followed by treatment with enzyme preparations from *Clostridium perfringens* resulted in 75% carbohydrate scission from the blood-group substance without alteration of the polypeptide chain. A

[246] P. Weber and R. J. Winzler, *Arch. Biochem. Biophys.*, 1969, **129**, 534.
[247] A. L. Tarentino and F. Maley, *Arch. Biochem. Biophys.*, 1969, **130**, 295.
[248] J. Conchie and I. Strachen, *Biochem. J.*, 1969, **115**, 709.
[249] E. Buddecke, H. Schauer, E. Werries, and A. Gottschalk, *Biochem. Biophys. Res. Comm.*, 1969, **34**, 517.
[250] A. Gottschalk, *Nature*, 1969, **222**, 452.
[251] L. M. Likhosherstov, V. A. Derevitskaya, and V. I. Fedorova, *Biokhimiya*, 1969, **34**, 45.
[252] L. M. Likhosherstov, M. D. Martynova, and V. A. Derevitskaya, *Biokhimiya*, 1968, **33**, 1135.

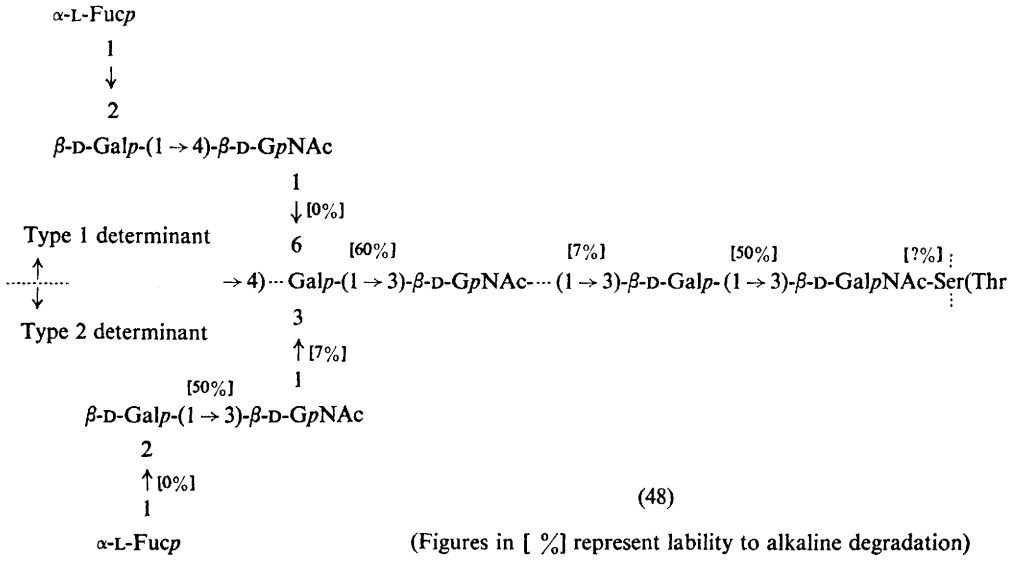
high-molecular-weight fragment contained 2-acetamido-2-deoxy-D-galactose (30%) and D-galactose (*ca.* 6%). The preponderance of 2-acetamido-2-deoxy-D-galactose and its loss during subsequent alkaline treatment provided evidence that the hexosamine was involved in the region of *O*-glycosidic carbohydrate–peptide linkage.[253] Methylation demonstrated that fucose and some of the galactose residues were present as terminal groups and the remainder of the galactose occurred within the chains with substituents at C-3, C-4, and C-6. Threitol was found amongst the Smith degradation products, confirming the C-4 substitution of the galactose residues.[254]

To assess the competition between reduction and elimination at each stage in the peeling of the carbohydrate chains from blood-group substances A, B, H, and Le^a by sodium hydroxide–sodium borohydride, some model oligosaccharides have been studied under identical conditions.[255] The extent of cleavage was determined by g.l.c. analysis of the trimethylsilyl derivatives of the eliminated products. *O*-β-D-Galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-glucose and *O*-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactose were found to be the most labile (*ca.* 50% cleavage) compared with *O*-β-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1 → 3)-D-galactose (*ca.* 7% cleavage). Two other oligosaccharides studied, *O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose and *O*-β-(2-acetamido-2-deoxy-D-glucopyranosyl)-(1 → 6)-D-galactose, were not degraded under these conditions. A simplified structure for the mono-L-fucopyranosyl chains of blood-group H substance was proposed, showing the lability of the various linkages to the alkaline reagent (48). These findings explained the observations that (*a*) type 2 oligosaccharides were found in greater amounts than type 1 oligosaccharides, and (*b*) the penultimate D-galactopyranose unit was converted into a hex-3-enetetrol by elimination of type-1 chain from C-3 and of an unknown group from C-4.

A blood-group substance has been isolated from ovarian cyst fluid which lacked A, B, H, Le^a, and Le^b specificities.[256] This material, suggested as a possible blood-group-substance precursor, gave a high cross-reaction with antipneumococcal type 14 serum, indicative of terminal galactose residues. The major difference in composition from the normal blood-group substances was the lack of L-fucose residues.

A tetrasaccharide common to MM, MN, and NN blood-group antigens has been isolated after alkaline borohydride cleavage of the carbohydrate–protein linkage.[257] Removal of two moles of *N*-acetyl-neuraminic acid by neuraminidase treatment gave a galactosyl-2-acetamido-2-deoxygalactitol. A tentative structure was suggested on the basis of periodate oxidation

[253] N. K. Kochetkov, V. A. Derevitskaya, L. M. Likhosherstov, and M. D. Martynova, *Doklady. Akad. Nauk. S.S.S.R.*, 1969, **186**, 1195.
[254] N. K. Kochetkov, G. S. Kykot', L. S. Bogdanova, L. M. Likhosherstov, and V. A. Derevitskaya, *Izvest. Akad. Nauk. S.S.S.R. Ser. khim.*, 1968, 2085.
[255] K. O. Lloyd and E. A. Kabat, *Carbohydrate Res.*, 1969, **9**, 41.
[256] G. Vicari and E. A. Kabat, *J. Immunol.*, 1969, **102**, 821.
[257] A. M. Adamany and R. H. Kathan, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 171.



(48)

(Figures in [%] represent lability to alkaline degradation)

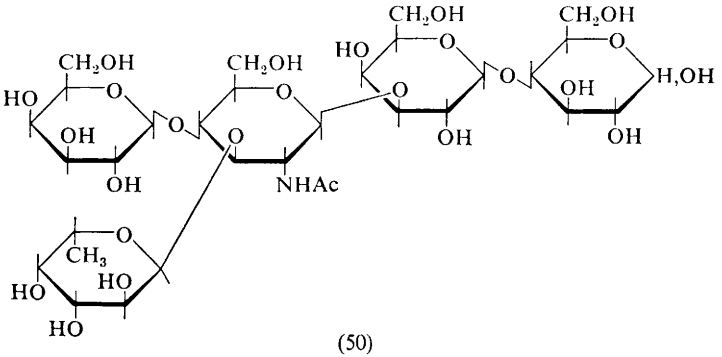
data (49). Degradation of *N*-acetyl-neuraminic acid-free blood-group M

N-acetyl NA *N*-acetyl NA
2 2
↓ ↓
2(4) 6
Gal*p*-(1 → 3)-D-Gal NAc(OH)

(49)

and N glycoproteins with alkaline borohydride gave galactosyl-2-acetamido-2-deoxy-galactitol, accounting for 80—100% of the low-molecular-weight galactose produced.[258] From the results of this study, it was concluded that the mannose, fucose, 2-acetamido-2-deoxy-glucose, and the rest of the galactose was present in alkali-stable-linked oligosaccharides.

A new pentasaccharide, lacto-*N*-fucopentaose III (50), has been isolated and characterised from human milk.[259] The oligosaccharide showed no



(50)

haptenic activity under conditions where lacto-*N*-fucopentaose II was active as a haptenic inhibitor of agglutination of Le^a red cells by Le^a antisera. The four oligosaccharides containing *O*-α-L-(1 → 2)-*O*-β-D-galactopyranosyl residues were found in milk from donors with blood type Le^b but not in those with blood type Le^a.[260]

The peptide moiety of human blood-group-active glycoprotein associated with the ABO and Lewis groups has been studied after removal of the carbohydrate by chemical and enzymic degradations.[261, 262] A blood-group-A-reactive haemagglutinin from *Helix pomatia* has been purified by

[258] E. Lisowska, *European J. Biochem.*, 1969, **10**, 574.
[259] A. Kobata and V. Ginsburg, *J. Biol. Chem.*, 1969, **244**, 5496.
[260] A. Kobata, V. Ginsburg, and M. Tsuda, *Arch. Biochem. Biophys.*, 1969, **130**, 509.
[261] A. S. R. Donald, J. M. Creeth, W. T. J. Morgan, and W. M. Watkins, *Biochem. J.*, 1969, **115**, 125.
[262] W. T. J. Morgan and W. M. Watkins, *Brit. Med. Bull.*, 1969, **25**, 30.

adsorption on polyleucyl-blood-group A substance and elution with a solution of 2-acetamido-2-deoxy-D-galactose.[263] Blood-group A substance precipitation was best inhibited by methyl 2-acetamido-2-deoxy-α-D-galactopyranoside. The purified haemagglutinin was also applicable to the determination of 2-acetamido-2-deoxy-α-D-galactopyranosyl residues in a mixture of α- and β-teichoic acids.

The formation of blood-group A substance from blood-group H substance and UDP-[^{3}H]-2-acetamido-2-deoxy-D-galactose by the action of a 2-acetamido-2-deoxy-α-D-galactopyranosyl transferase from hog gastric mucosa has been reported.[264] The properties of an α-D-galactosyl transferase from human tissues from blood-group B donors have been investigated.[265] Schemes have been postulated where α-L-fucosyl transferases specified by H and Le genes control the addition of L-fucose to different acceptor sites in a precursor to give H, Le^a, or Le^b serologically-active structures. The α-L-fucosyl transferases required for H-, Le^a-, and Le^b-specific structures have been found in tissues in which the blood-group glycoproteins were synthesised.[266]

Submaxillary Gland.—A large proportion of the disaccharide side-chains (85%) of bovine submaxillary mucin have been cleaved by a β-elimination reaction with alkali.[267] The peptide chain contained unsaturated amino-acids at most of the original threonine and serine sites. Hydrolytic scission of 2-aminopropenoic acid linkages occurred on heating at 100 °C for 1 h. at pH 2·2, but not at 2-amino-but-3-enoic acid residues. Cleavage of the *O*-glycosidic bond between the carbohydrate and polypeptide chains in bovine (and also ovine) submaxillary mucin has also been achieved with a 2-acetamido-2-deoxy-α-D-galactosidase from ox spleen.[268] This enzyme had absolute specificity for 2-acetamido-2-deoxy-α-D-galactopyranosyl linkages, in contrast to the established 2-acetamido-2-deoxy-β-D-glucosidase which did not distinguish between the differing stereochemistry at C-4.

An improved method has been described for the isolation and purification of porcine submaxillary mucins.[269] By this method two glycoprotein fractions were resolved. The major component contained D-galactose, 2-acetamido-2-deoxy-D-galactose, *N*-glycollyl-neuraminic acid, and L-fucose (1 : 1·89 : 0·76 : 0·80), whilst the minor component contained the same residues in the proportions 1 : 1·93 : 0·73 : 0·84.

The variation in the carbohydrate composition of submaxillary mucins and parotid saliva of the dog has been examined in relation to the intensity

[263] S. Hammerstrom and E. A. Kabat, *Biochemistry*, 1969, **8**, 2696.
[264] H. Tuppy and H. Schenkel-Brunner, *European J. Biochem.*, 1969, **10**, 152.
[265] C. Race and W. M. Watkins, *Biochem. J.*, 1969, **114**, 867.
[266] M. A. Chester and W. M. Watkins, *Biochem. Biophys. Res. Comm.*, 1969, **34**, 835.
[267] F. Downs and W. Pigman, *Biochemistry*, 1969, **8**, 1760.
[268] E. Werries, E. Wallek, A. Gottschalk, and E. Buddecke, *European J. Biochem.*, 1969, **10**, 445.
[269] M. De Salegui and H. Plonska, *Arch. Biochem. Biophys.*, 1969, **129**, 49.

of secretory stimulus.[270] The carbohydrate moiety of the parotid glycoprotein differed significantly from that of the submaxillary mucin principally in its increased galactose content. Salivary submaxillary mucin only contained traces of galactose. The variation in *N*-acetyl-neuraminic acid and L-fucose contents was attributed to the presence of different proportions of two glycoproteins.

The occurrence and partial purification of a 2-acetamido-2-deoxy-α-D-galactosidase from mammalian sources have been described.[271] This enzyme preparation released 2-acetamido-2-deoxy-D-galactose from bovine submaxillary mucin only after prior removal of the *N*-acetyl-neuraminic acid residues from the glycoprotein.

The preparation of glycosyl transferases[272] and a polypeptide : 2-acetamido-2-deoxy-D-galactose transferase[273] has been described.

Enzymes.—The homogeneity of a glycopeptide from Taka-amylase A has been reinvestigated by column chromatography and electrophoresis.[274] The glycopeptide contained D-mannose, 2-acetamido-2-deoxy-D-glucose, serine, aspartic acid, and ammonia (6 : 2 : 1 : 1 : 1) and was subjected to sequential Smith degradations. Application of specific glycosidase hydrolysis allowed a sequence of units to be deduced and the anomeric configurations of the linkages to be established (51).[275] The carbohydrate–peptide bond was confirmed as a 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosyl amine linkage.

$$\begin{array}{l} \text{M} \rightarrow \text{M} \rightarrow \text{M} \rightarrow \text{M} \rightarrow \text{GNAc} \rightarrow \text{GNAc} \rightarrow \text{(NH)Asp—Ser} \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \Big\downarrow [IO_4^-,\ BH_4^-,\ H^+] \times 2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \text{GNAc} \rightarrow \text{GNAc—(NH)Asp-CO·CH}_2\text{OH} \end{array}$$

(51)

A series of glycopeptides of the type ser-asp(NH-oligosaccharide) and asp(NH-oligosaccharide) were obtained by exhaustive proteolysis of *Aspergillus oryzae* α-amylase.[276] The oligosaccharide portions of the glycopeptides contained 2-acetamido-2-deoxy-D-glucose, mannose, galactose, arabinose, and xylose. Since the glycopeptides were resolved into a number of fractions with different carbohydrate composition, it was concluded that the enzyme was microheterogeneous with respect to the

[270] Z. Dische, C. McNeill Burgher, A. Danilchenko, and C. Rothschild, *Arch. Biochem. Biophys.*, 1969, **135**, 1.
[271] B. Weissmann and D. F. Hinrichsen, *Biochemistry*, 1969, **8**, 2034.
[272] A. Hagopian and E. H. Eylar, *Arch. Biochem. Biophys.*, 1969, **129**, 447.
[273] A. Hagopian and E. H. Eylar, *Arch. Biochem. Biophys.*, 1969, **129**, 515.
[274] H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1969, **65**, 793.
[275] H. Yamaguchi, T. Mega, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1969, **66**, 441.
[276] J. F. McKelvy and Y. C. Lee, *Arch. Biochem. Biophys.*, 1969, **132**, 99.

carbohydrate chains. Comparative o.r.d. measurements of the acid-stable and -unstable forms of *A. niger* α-amylase indicated that there were no major differences in the folding of the polypeptide chains.[277]

Invertase attached externally to cell membranes, in contrast to the intracellular enzyme, contains *ca.* 50% carbohydrate, largely in the form of mannose with small amounts of 2-acetamido-2-deoxy-glucose.[278] Study of derived glycopeptides has provided evidence for a glucosaminyl–asparagine linkage with a possible small proportion of the *O*-seryl–manno-side-type linkage. The carbohydrate–peptide bond in β-fructofuranosidase has been investigated.[279]

Collagens.—Di- and tri-saccharides accounting for 90% of the D-galactose content of earthworm (*Lumbricus terrestris*) collagen were obtained on treatment with sodium hydroxide–sodium borohydride.[280] Structural studies indicated that the disaccharide was 2-*O*-(α-D-galactopyranosyl)-D-galactose and the trisaccharide *O*-α-D-galactopyranosyl-(1 → 2)-*O*-α-D-galactopyranosyl-(1 → 2)-D-galactose.

Pronase digestion of several collagens gave a series of hydroxylysine-containing glucogalactanpeptides.[281] Alkaline hydrolysis resulted in the formation of glucosyl-galactosyl-hydroxylysine and a galactosyl-hydroxylysine. Analysis of the glycopeptide obtained from collagenase–pronase digestion of ichthyocol indicated the presence of 2-*O*-(α-D-glucopyranosyl)-β-D-galactosyl residues.

The variation in carbohydrate content of human and bovine polymeric collagens from various tissues has been investigated.[282] Although the 2-amino-2-deoxy-hexose content was uniform (*ca.* 1 unit per 3000 amino-acid units) the content of galactose, glucose, mannose, and fucose was widely variable. It was suggested that these sugar units played an important role in the polymerisation and/or the differentiation of collagen in structural tissues. The carbohydrate content was inversely related to the mean collagen fibre diameter. Fractionation of the collagen–protein–polysaccharide complex of human intervertebral discs was achieved by cetyltrimethylammonium bromide–cellulose chromatography.[283] Collagen was separated from a protein–polysaccharide complex. It was concluded that the stability of the collagen-containing complex was due to ionic interactions between the amino-groups on collagen and an acidic function on the protein–polysaccharide complex.

[277] T. Takagi, M. Arai, Y. Monoda, T. Isemura, and K. Yameda, *Biochim. Biophys. Acta*, 1969, **175**, 438.
[278] N. P. Neumann and J. O. Lampen, *Biochemistry*, 1969, **8**, 3552.
[279] H. Greiling, P. Vogele, R. Kisters, and H. D. Ohlenbusch, *Z. physiol. Chem.*, 1969, **350**, 517.
[280] L. Muir and Y. C. Lee, *J. Biol. Chem.*, 1969, **244**, 2343.
[281] R. G. Spiro, *J. Biol. Chem.*, 1969, **244**, 602.
[282] M. E. Grant, I. L. Freeman, J. D. Scholfield, and D. S. Jackson, *Biochim. Biophys. Acta*, 1969, **177**, 682.
[283] F. S. Steven, J. Knott, and D. S. Jackson, *Biochim. Biophys. Acta*, 1969, **188**, 307.

Analysis of the collagen from *Thyone briareus* (echinoderm) invertebrate indicated that glucose and galactose were the sole carbohydrate residues present.[284]

The solubilisation of collagen complex from developing lathyritic chick cartilage [285] and the effect of ascorbic acid on guinea pig skin collagen synthesis [286] have been studied.

Serum Glycoproteins.—Possible complex formation between polysaccharides and albumin or IgG has been investigated by chromatography of albumin and IgG on columns of dextran, hyaluronic acid, and chondroitin 4-sulphate.[287] The results of these studies and equilibrium dialysis investigation of the partition between albumin or IgG and hyaluronic acid indicated that complex formation did not occur in phosphate buffer (0·05 M, pH 7·4) containing sodium chloride (0·1M).

Analysis of the immunoglobulin light chain produced and secreted by plasma tumour in mice (MOPC 46) indicated that the glycoprotein was unusual in having 12% carbohydrate attached to either asparagine or aspartic acid.[288] Residue 28 (Mouse 41 numbering system) was suggested as the site of carbohydrate attachment.

The proteolysis of human, rabbit, and bovine IgG by papain was considered to be controlled by the distribution of the carbohydrate chains along the polypeptide chain.[289]

A glycopeptide from human α_1-acid-glycoprotein, orosomucoid, has been isolated after neuraminidase and pronase digestion by chromatography on sulphoethyl cellulose.[290] The general structural features of the glycopeptide were established by periodate oxidation studies and degradation with specific glycosidases. The nature of the carbohydrate–peptide bond was established by the isolation of 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose. A partial structure for the glycopeptide was postulated (52) on the basis of this evidence. Thermal polymerisation of orosomucoid balls (containing 8 monomer units) and chains under conditions of different ionic strengths has been studied.[291] Removal of the *N*-acetyl-neuraminic acid residues allowed only chain polymers to be formed. The effect of these polymers on influenza virus haemagglutination has been reported.[292]

[284] R. L. Katzman, A. K. Bhattacharyya, and R. W. Jeanloz, *Biochim. Biophys. Acta*, 1969, **184**, 523.
[285] M. J. Glimcher, J. Seyer, and D. M. Brickley, *Biochem. J.*, 1969, **115**, 923.
[286] V. Richmond and E. L. R. Stokstad, *J. Dental Res.*, 1969, **48**, 83.
[287] K. Hellsing, *Biochem. J.*, 1969, **112**, 483.
[288] F. Melchers, *Biochemistry*, 1969, **8**, 938.
[289] R. B. Payne, *Biochem. J.*, 1969, **111**, 473.
[290] P. V. Wagh, I. Bernstein, and R. J. Winzler, *J. Biol. Chem.*, 1969, **244**, 658.
[291] S. P. Spragg, H. B. Halsall, T. H. Flewett, and G. R. Barclay, *Biochem. J.*, 1969, **111**, 345.
[292] G. R. Barclay, T. H. Flewett, E. Keller, H. B. Halsall, and S. P. Spragg, *Biochem. J.*, 1969, **111**, 353.

β-D-Galp
1
↓
4(3)
β-D-GpNAc
1
↓
2(4)
β-D-Galp-(1→4 or 3)-β-D-GpNAc-(1→3)-α-D-Mp
1
↓
3(4 or 6)
α-D-Mp-(1→3 or 4)-β-D-GpNAc-(1→2 or 4)-D-Mp-(1→3 or 4 or 6)-D-GpNAc→Asparagine
3
↑
1
β-D-GpNAc
4(3)
↑
1
β-D-Galp

(52)

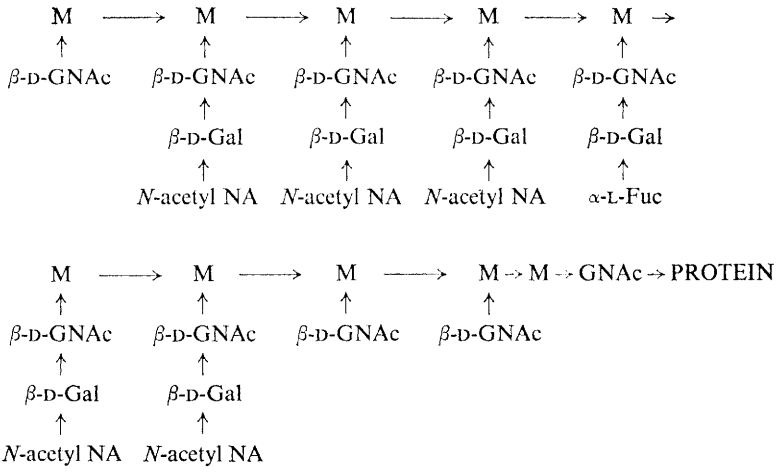
A sialic-acid-free β_1-glycoprotein has been isolated from human plasma [293] containing 30% carbohydrate. Analytical studies indicated the presence of mannose, galactose, fucose, and 2-acetamido-2-deoxy-glucose (15, 13, 2, and 22 residues per molecular weight of 31,000). The isolation of five glycopeptide fractions with differing carbohydrate analyses (particularly with respect to the ratio of fucose, mannose, and galactose) suggested that polymorphism of α_1-acid glycoprotein was due to variation in the carbohydrate composition.[294]

Miscellaneous Glycoproteins—Human chorionic gonadotropin (HCG) has been purified by alcohol precipitation, ion-exchange chromatography, and gel permeation chromatography.[295] The preparation was homogeneous with respect to size and amino-acid composition but appeared to be heterogeneous with respect to electrical charge and sialic acid content. Furthermore, the glycoprotein consisted of two seemingly identical subunits, each of a molecular weight in the range 25,000—28,000. Bahl [296] has reported analytical and structural studies of HCG based on a molecular weight of 27,000. The carbohydrate portion of the glycoprotein (31·3%) was composed of galactose (9), mannose (9), fucose (1), 2-acetamido-2-deoxy-D-glucose (11), 2-acetamido-2-deoxy-D-galactose (3), and sialic acid (8 residues per 27,000 molecular weight). After removal of the sialic acid, reduction with 2-mercaptoethanol, and carboxamidomethylation with iodoacetamide, tryptic hydrolysis gave two glycopeptides.[297] Analysis of the carbohydrate composition indicated that these were distinct with respect to structure, and sequential treatment with specific glycosidases allowed sequences of carbohydrate residues to be postulated (53).

Periodate oxidation studies of human pituitary follicle stimulating hormone (FSH) have been reported.[298] Periodate oxidation followed by borohydride reduction and acid hydrolysis gave propan-1,2-diol but no threitol or erythritol. Determination of the sugar units resistant to oxidation indicated that galactose (59%), mannose (80%), and 2-acetamido-2-deoxy-glucose (87%) had been oxidised in addition to fucose and *N*-acetylneuraminic acid. Radioimmunological assay of the fragments produced suggested that the carbohydrate was not involved in the immunological activity of the hormone, although essential for manifestation of biological activity.

A method for the preparation of ovine FSH of high biological purity by recycling chromatography on a gel permeation column has been described.[299] The resultant hormone, however, still appeared to be

[293] J. Labat, M. Ishiguro, Y. Fujisaki, and K. Schmid, *J. Biol. Chem.*, 1969, **244**, 4975.
[294] T. Yamaguchi and I. Yamashina, *J. Biochem. (Japan)*, 1969, **66**, 213.
[295] J. J. Bell, R. E. Ganfield, and J. J. Sciarra, *Endocrinol.*, 1969, **84**, 298.
[296] O. P. Bahl, *J. Biol. Chem.*, 1969, **244**, 567.
[297] O. P. Bahl, *J. Biol. Chem.*, 1969, **244**, 575.
[298] J. F. Kennedy and W. R. Butt, *Biochem. J.*, 1969, **115**, 225.
[299] C. Hermier and M. Jutisz, *Biochim. Biophys. Acta*, 1969, **175**, 402.



(53)

heterogeneous. Sulphosalicylic acid has been used to aid the purification of urinary FSH.[300]

Bovine lutenising hormone (LH) has been resolved into two sub-units, LH C-1 and LH C-2, by countercurrent distribution.[301] Both LH C-1 and C-2 appeared to have a molecular weight of 14,609 compared with a value of 29,258 estimated for the original LH. The carbohydrate and amino-acid compositions of the two sub-units were non-identical, LH C-2 containing fucose, mannose, 2-acetamido-2-deoxy-glucose, and 2-acetamido-2-deoxy-galactose in the proportions 0·063, 0·197, 0·188, and 0·083 mmoles mg^{-1}, compared with 0·055, 0·566, 0·293, and 0·104 for the constituents of LH C-1. The LH C-1 sub-unit had approximately ten times the biological and immunological activity of the LH C-2 sub-unit, and a threefold synergistic effect on the biological activity on reassociation in 1 : 1 molar proportions was observed.

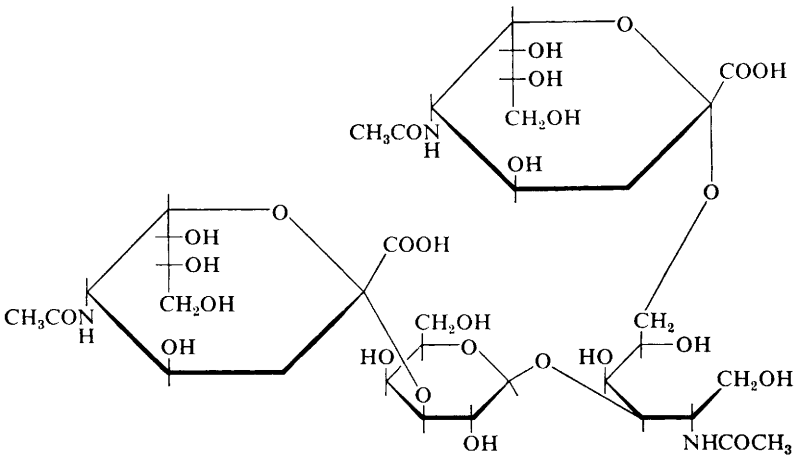
Countercurrent distribution has also been employed to obtain a purified fraction of bovine thyroid-stimulating hormone (TSH).[302] This method removed persistent impurities including one of the chains of LH. No evidence for chain separation was obtained and a molecular weight of 25,000 was estimated. Rechromatographed TSH contained fucose (0·9), mannose (6·9), galactose (0·3), 2-acetamido-2-deoxy-glucose (7·8), and 2-acetamido-2-deoxy-galactose (3·3 residues per 25,000 molecular weight). Although TSH and LH did not cross-react with antisera prepared against

[300] M. Theoleyre and M. M. Jutisz, *Compt. rend.*, 1969, **268**, *D*, 1994.
[301] L. E. Reichert jun., M. A. Rasco, D. N. Ward, G. D. Niswender, and A. R. Midgely jun., *J. Biol. Chem.*, 1969, **244**, 5110.
[302] T. H. Liao, G. Hennen, S. M. Howard, B. Shome, and J. G. Pierce, *J. Biol. Chem.*, 1969, **244**, 6458.

the native form of the other hormone, antisera prepared against LH C-1 cross-reacted with TSH, in addition to its reaction with LH.

Sulphitolysis of human fibrinogen resulted in the formation of three glycopeptides.[303]

Oligosaccharides have been obtained from M- and N-active sialoglycopeptides of human erythrocytes by treatment with alkaline sodium borohydride.[304, 305] No differences were observed between the oligosaccharide fragments from the M- and N-active glycopeptides. A branched tetrasaccharide (54) containing 2 moles of *N*-acetyl-neuraminic acid was

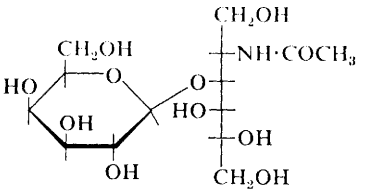


(54)

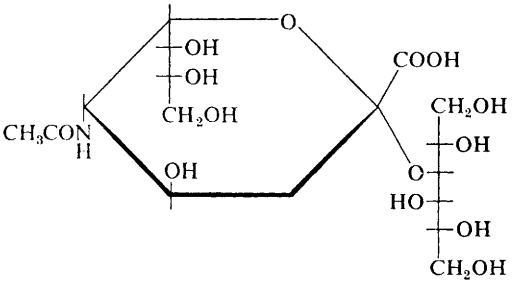
isolated. This was considered to be the major alkali-labile oligosaccharide, the other observed fragments, two disaccharides (55, 56) and various monomeric units, being derived from the tetramer. The linkage to the peptide chain appeared to be *via* an *O*-(2-acetamido-2-deoxy-D-galactosyl)serine moiety. Fractionation of sialoglycopeptides from human platelet membranes resulted in the isolation of six glycopeptides.[306] All fractions contained *N*-acetyl-neuraminic acid, 2-acetamido-2-deoxy-glucose, mannose, and galactose, whilst four fractions contained glucose in addition. No alkali-labile *O*-glycosylserine linkages were detected.

A gastroferrin has been isolated from human gastric juice by co-precipitation of an iron–gastroferrin complex with ferric hydroxide.[307] This glycoprotein (molecular weight 260,000) had a high carbohydrate content

[303] D. A. Millo and D. C. Triantaphyllopoulos, *Arch. Biochem. Biophys.*, 1969, **135**, 28.
[304] D. B. Thomas and R. J. Winzler, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 811.
[305] D. B. Thomas and R. J. Winzler, *J. Biol. Chem.*, 1969, **244**, 5943.
[306] D. S. Pepper and G. A. Jamieson, *Biochemistry*, 1969, **8**, 3362.
[307] P. J. Davis, J. S. Multani, C. P. Cepurneek, and P. Saltman, *Biochem. Biophys. Res. Comm.*, 1969, **37**, 533.



(55)



(56)

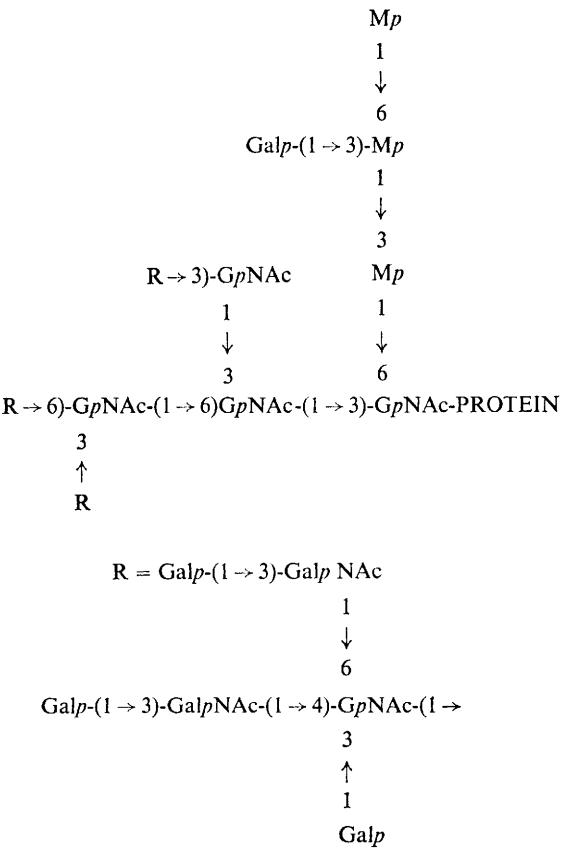
(85%) composed of units of 2-acetamido-2-deoxy-glucose, 2-acetamido-2-deoxy-galactose, galactose, fucose, and *N*-acetyl-neuraminic acid.

A water-soluble fraction of porcine gastric mucosa contained a muco-protein with both A and H blood-group activity.[308] Study of *in vitro* incorporation of D-[U-^{14}C]glucose revealed predominant incorporation into the galactose residues of the glycoprotein (77% of incorporated activity) with smaller incorporation into fucose (5%) and 2-amino-2-deoxy-hexose (8%).

2-Acetamido-2-deoxy-glyceraldehyde diethyl dithioacetal has been isolated from the Smith degradation products of an ovalbumin glycopeptide after mercaptolysis.[309] Extensive degradation of ovalbumin with pronase gave glycopeptides rich in aspartic acid with small quantities of leucine.[310] The derived glycopeptides, in contrast to the original ovalbumin, were degraded by 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose amidohydrolase, indicating the nature of the carbohydrate–peptide bond. Action of an α-D-mannosidase and a 2-acetamido-2-deoxy-β-D-glucosidase on either ovalbumin or the glycopeptides suggested that two mutually linked 2-acetamido-2-deoxy-D-glucose units were present at the reducing termini, attached to an α-linked D-mannose unit. Pronase digestion of ovomucoid gave four glycopeptides containing *N*-acetyl-neuraminic acid (1), 2-acetamido-2-deoxy-glucose (14—20), and hexose (11—21 units).[311]

[308] D. Snory and A. Allen, *Biochem. J.*, 1969, **114**, 85P.
[309] S. David and A. Veyrieres, *Carbohydrate Res.*, 1969, **10**, 35.
[310] J. Conchie, A. J. Hay, I. Strachan, and G. A. Levy, *Biochem. J.*, 1969, **115**, 717.
[311] M. Kanamori and M. Kawabata, *Agric. and Biol. Chem. (Japan)*, 1969, **33**, 220.

Periodate oxidation studies of a bovine bone sialoprotein supported the conclusion that this material consisted of a small number (possibly only one) of highly branched oligosaccharide structures.[312] The only groups oxidised by periodate were the terminal *N*-acetyl- and *N*-glycollyl-neuraminic acid residues. Subsequent successive Smith degradation sequences indicated that 2-acetamido-2-deoxy-D-glucose was present in an inner part of the structure adjacent to the carbohydrate–peptide linkage. A possible highly branched structure was postulated (57) for the carbohydrate prosthetic group after removal of sialic acid. Acidic glycosaminoglycans and



(57)

glycopeptides have been reported in the organic matrix of embryonic bovine enamel.[313]

[312] A. T. de B. Andrewa, G. M. Herring, and P. W. Kent, *Biochem. J.*, 1969, **111**, 621.
[313] J. Seyer and M. J. Glimcher, *Biochem. Biophys. Acta*, 1969, **184**, 509.

Bovine colostrum M-1 glycoprotein has been resolved into two components of molecular weights 7200 (24·8% carbohydrate) and 12,000 (39% carbohydrate).[314] Oligosaccharide chains were liberated on alkaline borohydride treatment from both components. Provisional periodate oxidation data indicated that the terminal *N*-acetyl-neuraminic acid residues were attached to galactose residues probably *via* a (2 → 3) ketosidic linkage.

The sugar and amino-acid composition of calf and cow lens capsules has been examined.[315] Glycopeptides containing *O*-α-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-hydroxylysine were isolated[316] from this source.

Conditions of methanolysis have been established for the recovery and identification of 2-acetamido-2-deoxy-D-galactose from bovine κ-casein, thus providing proof for the presence of the *N*-acetyl substituent.[317]

Comparative studies have been reported on the Lorenzini jelly from *Raja clavata* and *Cetorhinus maximus*.[318] Sulphated glycopeptides were obtained containing D-galactose, 2-amino-2-deoxy-glucose and -galactose, and fucose. Fractionation and quantitative analyses revealed that, for each species, a range of glycopeptides were present varying from those with high 2-amino-2-deoxy-galactose plus peptide with low sulphate and 2-amino-2-deoxy-glucose contents to glycopeptides with low 2-amino-2-deoxy-galactose plus peptide with high sulphate and 2-amino-2-deoxy-glucose contents. It was not clear if the continuous variation in composition was a result of the use of pooled samples or material from different sites in the organ.

Removal of the sialic acid from fetuin did not significantly alter the slightly elongated but compact molecular conformation.[319] Other workers[320] have studied the sequential release of monosaccharides from fetuin and fetuin glycopeptides by lysosomal glycosidases.

The compositional patterns of sialofucohexosaminoglycans derived by proteolysis from rat brain glycoproteins have been examined.[321] Six fractions were obtained by column electrophoresis which were characterised by their increasing *N*-acetyl-neuraminic acid and decreasing fucose contents. All the fractions also contained galactose, mannose, 2-amino-2-deoxy-glucose, and 2-amino-2-deoxy-galactose.

The protein–polysaccharide complexes secreted *in vitro* by calf tracheal epithelium have been separated by gel permeation chromatography.[322]

[314] A. Bezkorovainy and D. Grohlich, *Biochem. J.*, 1969, **115**, 817.
[315] S. Fukushi and R. G. Spiro, *J. Biol. Chem.*, 1969, **244**, 2041.
[316] R. G. Spiro and S. Fukushi, *J. Biol. Chem.*, 1969, **244**, 2049.
[317] J. V. Wheelock and G. Sinkinson, *Biochim. Biophys. Acta*, 1969, **194**, 597.
[318] M. J. How, J. V. S. Jones, and J. Doyle, *Carbohydrate Res.*, 1969, **11**, 207.
[319] Y. Oshiro and E. H. Eylar, *Arch. Biochem. Biophys.*, 1969, **130**, 227.
[320] S. Mahadevan, C. J. Dillard, and A. L. Tappel, *Arch. Biochem. Biophys.*, 1969, **129**, 525.
[321] C. Di Benedetta, E. G. Brunngraber, G. Whitney, B. D. Brown, and A. Aro, *Arch. Biochem. Biophys.*, 1969, **131**, 404.
[322] P. W. Kent, I. J. Molineux, M. R. Haegela, and F. K. Stevenson, *Biochem. J.*, 1969, **114**, 87P.

Complex A, which was devoid of serum components, contained galactose (46 residues per $2{\cdot}5 \times 10^5$ molecular weight), mannose (15), fucose (5—12), xylose (9—13), glucuronic acid (48), 2-acetamido-2-deoxy-glucose (23), 2-acetamido-2-deoxy-galactose (41), and sialic acid (5·3) and was an inhibitor of haemagglutination by influenza B virus. Complexes B and C contained 9·2 and 6·2% carbohydrate respectively with a similar monomer composition but were not inhibitory.

Virus transformation of mouse fibroplasts resulted in a lowering of the content of most neutral and amino-sugars, particularly *N*-acetyl-neuraminic acid and 2-acetamido-2-amino-galactose, in the membranes.[323]

A crystalline glycopeptide has been recovered from normal human urine.[324] Analysis indicated the presence of glucuronic acid (1 unit), 2-amino-2-deoxy-glucose and -galactose (1), galactose and xylose (10), together with serine, glycine, alanine, glutamic acid, and aspartic acid, and the absence of sulphate.

Collocalia mucoid contained a sialic acid (9%) provisionally identified as *N*-acetyl-4-*O*-acetyl-neuraminic acid.[325] In addition, fucose (0·7%), galactose (16·9%), 2-amino-2-deoxy-galactose (7·2%), and 2-amino-2-deoxy-glucose (5·3%) were determined.

Further structural studies on soybean haemagglutinin[326] have shown that 1-L-β-aspartamido-(2-acetamido)-1,2-dideoxy-β-D-glucose represented the carbohydrate–peptide linkage region. The isolation of a glycopeptide from a 7s protein in soybean globulins by proteolysis has provided further evidence for the glycoprotein nature of globulin.[327] The glycopeptide contained seventeen amino-acid residues and fifty-one carbohydrate units (thirty-nine mannose, twelve 2-amino-2-deoxy-glucose), and the correspondence of the composition to that of the original 7s globulin suggested that the carbohydrate moiety was present as a single polysaccharide unit.

The two major components of pineapple bromelain appeared to have the same oligosaccharide group composed of 2-acetamido-2-deoxy-D-glucose (2 units), D-mannose (2), D-xylose (1), and L-fucose (1).[328] Methylation and periodate oxidation data indicated a highly branched structure, possibly consisting of a main chain of mutually joined 2-acetamido-2-deoxy-D-glucose residues substituted by two residues of the three neutral sugar components.

Suspension cultures of tomatoes gave a series of hydroxyproline-rich glycopeptides.[329] Hydroxyproline-*O*-arabinosides were isolated, indicating the nature of the carbohydrate–peptide linkage region.

[323] H. C. Wu, E. Meezan, P. H. Black, and P. W. Robbins, *Biochemistry*, 1969, **8**, 2509; E. Meezan, H. C. Wu, P. H. Black, and P. W. Robbins, *Biochemistry*, 1969, **8**, 2518.
[324] D. Basu, *Biochem. J.*, 1969, **112**, 379.
[325] R. H. Kathan and D. I. Weeks, *Arch. Biochem. Biophys.*, 1969, **134**, 572.
[326] H. Lis, N. Sharon, and E. Katchalski, *Biochim. Biophys. Acta*, 1969, **192**, 364.
[327] I. Koshiyama, *Arch. Biochem. Biophys.*, 1969, **130**, 370.
[328] J. Scocca and Y. C. Lee, *J. Biol. Chem.*, 1969, **244**, 4852.
[329] D. T. A. Lamport, *Biochemistry*, 1969, **8**, 1155.

Pepsinogen contained a covalently bound sugar (D-glucose) in contrast to pepsin prepared from the same pepsinogen.[330]

The allergen from short ragweed pollen contained hexose (4·9%), pentose (8·7%), and hexosamine (0·8%).[331]

[330] H. Neumann, U. Zehavi, and T. D. Tanksley, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 1151.

[331] B. J. Underdown and L. Goodfriend, *Biochemistry*, 1969, **8**, 980.

6
Acidic Glycosaminoglycans

Polysaccharide Sulphates and Hyaluronic Acid from Animal Tissues

Further reports have appeared on the finding of sulphated glycosaminoglycans in a wide range of animal tissues including bovine foetal articular, epiphyseal plate, and costal cartilage,[332] porcine laryngeal cartilage,[333] cystic fibroplasts,[334] human term placenta,[335] peripheral nerve and spinal cord of monkey,[336] rabbit lungs,[337] human [338] and animal aorta,[339] and squid skin.[340] The differential effects of hypophysectomy on skin and tracheal cartilage glycosaminoglycans has been investigated.[341] Age effects on acidic glycosaminoglycan distribution and composition have been studied in various tissues including human aorta,[342] human bronchial cartilage,[343] and porcine femoral articular cartilage.[344] The phylogenetic aspects of acidic glycosaminoglycans in invertebrate connective tissue have been discussed.[345] A correlation has been observed between the fixed negative charge with acidic glycosaminoglycan content of human articular cartilage.[346]

Study of aspects of the isolation of connective tissue proteoglycans led to the conclusion that, even after repeated reprecipitation with cetylpyridinium chloride, residual non-covalently bound protein was present.[347]

[332] R. D. Campo, C. D. Tourellotte, and R. J. Bielen, *Biochim. Biophys. Acta*, 1969, **177**, 501.
[333] C. P. Tsiganas and H. Muir, *Biochem. J.*, 1969, **113**, 879, 885.
[334] B. S. Danes and A. G. Bean, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 919.
[335] A. Calatroni and N. diFerrante, *Carbohydrate Res.*, 1969, **10**, 535.
[336] E. V. Chandrasekaran and B. K. Backhawat, *J. Neurochem.*, 1969, **16**, 1529.
[337] I. A. Tseveleva, *Biokhimiya*, 1969, **34**, 459.
[338] E. Kimoto, K. Akiyama, and Y. Noguchi, *J. Biochem. (Japan)*, 1969, **66**, 369.
[339] R. N. Mullinger and G. Manley, *J. Atherosclerosis Res.*, 1969, **9**, 108.
[340] S. R. Sprinivasan, B. Radhakrishnamurthy, E. R. Dalferes jun., and G. S. Berenson, *Comp. Biochem. and Physiol.*, 1969, **28**, 169.
[341] J. A. Kolfoed and C. E. Bozzini, *Experientia*, 1969, **25**, 23.
[342] G. Manley, R. N. Mullinger, and P. H. Lloyd, *Biochem. J.*, 1969, **114**, 89.
[343] R. M. Mason, K. S. Dodgson, and F. S. Wusteman, *Biochem. J.*, 1969, **113**, 30P.
[344] K. D. Brandt and H. Muir, *Biochem. J.*, 1969, **114**, 871.
[345] R. L. Katzman and R. W. Jeanloz, *Science*, 1969, **166**, 758.
[346] A. Maroudas, H. Muir, and J. Wingham, *Biochim. Biophys. Acta*, 1969, **177**, 492.
[347] U. Lindhal, *Biochem. J.*, 1969, **113**, 569.

High and low shear procedures for the extraction of the protein–polysaccharide complex of bovine nasal cartilage have been compared [348] and the physical characterisation of protein–polysaccharide fractions from the same source by density-gradient sedimentation has been reported.[349] The function of glycoproteins in the formation of aggregates of these complexes has been discussed.[350]

The electrophoretic heterogeneity of the protein–polysaccharides from human costal cartilage, intervertebral discs, and bovine nasal septum cartilage has been investigated.[351]

The composition and immunological reactions of the sulphated mucopolysaccharides from chick chorioallantoic fluid have been examined.[352] The possible role of *N*-acetyl-neuraminic acid, L-fucose, and protein in the antigenic properties of these macromolecules was investigated.[352, 353]

A glycopeptide and three acidic glycosaminoglycan-peptides have been isolated from protease-digested gastric mucosa of rabbit.[354] Analytical studies revealed that all were highly sulphated and contained carbohydrate–peptide linkages of the *O*-seryl(threonyl)-glycoside type.[355]

The problems of extraction of acidic glycosaminoglycans from human skin have been overcome and the presence of three hyaluronic acid-containing fractions, chondroitin 4- and 6-sulphates, and dermatan sulphate demonstrated.[356] A scheme for the identification of the acidic glycosaminoglycans of skin based on the incorporation of general and specific precursors has been suggested.[357] L-Iduronic acid-[6-^{14}C], D-glucuronic acid-[U-^{14}C], and 2-(acetamido-[1-^{14}C])-2-deoxy-D-galactose were incorporated principally into dermatan sulphate, hyaluronic acid, and chondroitin sulphates respectively. The generally low incorporation into keratan sulphate was improved by use of D-galactose-[6-^{14}C].

Structural Studies

General Analytical Procedures.—Several improvements in the method for electrophoretic separation of acidic glycosaminoglycans have been reported. Use of cellulose acetate sheet electrophoresis in veronal buffers,[358] zinc acetate buffers,[359] and pyridyl formate or zinc acetate [360] have been

[348] S. W. Sajdera and V. C. Hascall, *J. Biol. Chem.*, 1969, **244**, 77.
[349] J. R. Dunstone and M. D. Franek, *J. Biol. Chem.*, 1969, **244**, 3654.
[350] V. C. Hascall and S. W. Sajdera, *J. Biol. Chem.*, 1969, **244**, 2384.
[351] V. Pedrini, *J. Biol. Chem.*, 1969, **244**, 1540.
[352] L. T. Lee, C. Howe, K. Meyer, and H. U. Choi, *J. Immunol.*, 1969, **102**, 144.
[353] K. Mortensson-Egnund, R. Schoyen, C. Howe, L. T. Lee, and A. Harboe, *J. Bacteriol.*, 1969, **98**, 924.
[354] T. Nemoto and Z. Yosizawa, *J. Biochem. (Japan)*, 1969, **66**, 627.
[355] T. Nemoto and Z. Yosizawa, *Biochim. Biophys. Acta*, 1969, **192**, 37.
[356] S. A. Barker, J. F. Kennedy, and P. J. Somers, *Carbohydrate Res.*, 1969, **10**, 57.
[357] S. A. Barker, J. F. Kennedy, and C. N. D. Cruickshank, *Carbohydrate Res.*, 1969, **10**, 65.
[358] A. Kimura and K. Tsurumi, *J. Biochem. (Japan)*, 1969, **65**, 303.
[359] R. E. S. Prout, *Biochim. Biophys. Acta*, 1969, **177**, 157.
[360] A. Gardais, J. Picard, and C. Tarasse, *J. Chromatog.*, 1969, **42**, 396.

variously used to fractionate different mixtures of the acidic glycosaminoglycans. A technique for the radiochemical determination of these polysaccharides after electrophoretic separation has been described.[361]

The technique of fractionation on Deacidite FF CO_3^{2-} anion exchange resin has been critically evaluated with respect to the separation of the acidic glycosaminoglycans of [^{35}S]sulphate-labelled cartilage.[362] The overall recovery of ^{35}S from the column was low (35—40%) and the factors influencing this were investigated. It was shown that complete separation was not possible, due to (*a*) steric heterogeneity of the resin sites and (*b*) slow elution of a portion of the adsorbed material. Consequently rigid chemical characterisation of eluted components from such systems was considered to be important and the use of the technique for quantitative assessment of labelled acidic glycosaminoglycans was questioned.

A spectrophotometric method for the determination of acidic glycosaminoglycans based on the shift in the absorption spectrum of a carbocyanine dye when bound to a polyanion has been described.[363] The o.r.d. spectra of acidic glycosaminoglycans and their dye complexes have been further studied.[364] Pulse radiolysis and spectral studies have been used to investigate the interaction of cetylpyridinium chloride and methylene blue complexes with connective tissue polyanionic glycosaminoglycans.[365]

Turbidimetric methods based on interaction with cationic detergents have been utilised for the determination and classification of the acidic glycosaminoglycans.[366–368]

A method has been reported for the determination of the molecular weight distribution of chondroitin sulphate on the microgram level by gel permeation chromatography on a column precalibrated with fractions of known molecular weight.[369]

Chondroitin Sulphates.—The sulphation of chondroitin sulphate in embryonic chick cartilage epiphyses has been investigated[370] by use of a purified chondroitinase preparation from *Proteus vulgaris*. Classification of the unsaturated disaccharides produced on enzyme degradation enabled the determination of non-sulphated chondroitin, chondroitin 4-sulphate, and chondroitin 6-sulphate to be made.

Degradation of the oversulphated chondroitin sulphate from human aortic tissue with enzyme preparations from *P. vulgaris* and *Flavobacterium*

[361] K. U. Paunio and K. K. Makinen, *J. Chromatog.*, 1969, **39**, 96.
[362] M. J. How, P. J. Wood, and C. N. D. Cruickshank, *Carbohydrate Res.*, 1969, **11**, 103.
[363] R. D. Edstrom, *Analyt. Biochem.*, 1969, **29**, 421.
[364] A. L. Stone, *Biopolymers*, 1969, **7**, 173.
[365] J. V. Davies, K. S. Dodgson, J. S. Moore, and G. O. Phillips, *Biochem. J.*, 1969, **113**, 465.
[366] P. D. Mier and M. Wood, *Clinica Chim. Acta*, 1969, **24**, 105.
[367] R. Bohn, V. Dinmendahl, and D. A. Kalbhen, *Z. analyt. Chem.*, 1969, **247**, 312.
[368] S. Y. Ali, *Biochim. Biophys. Acta*, 1969, **177**, 641.
[369] A. Wasteson, *Biochim. Biophys. Acta*, 1969, **177**, 152.
[370] H. C. Robinson and A. Dorfman, *J. Biol. Chem.*, 1969, **244**, 348.

heparinum has been reported.[371] A disulphated repeating unit was indicated, with a 2- or 3-sulphated hexuronic acid and a 6-sulphated 2-acetamido-2-deoxy-D-galactosamine unit.

A chondroitin sulphate proteoglycan has been isolated from human leukocyte granules.[372] Serine-[^{14}C] incorporated into the protein moiety of the proteoglycan was removed on treatment with alkali by a β-elimination reaction, indicating the nature of the carbohydrate–peptide linkage. Inhibition of [^{35}S]sulphate incorporation by puromycin indicated that polysaccharide chain extension was dependent on prior synthesis of protein. Other workers[373] have shown that the alkali-stable fraction of chondromucoprotein incorporated [^{35}S]sulphate and [^{14}C]-D-glucose more readily than the serine-linked moiety. An undersulphated polysaccharide was apparently concentrated in the alkali-stable portion. Hydrazinolysis of this material resulted in the formation of a polysaccharide containing glutamic acid as the major residual amino-acid, suggesting the presence of a linkage other than one involving serine.

A modified method has been reported for the determination of chondroitin sulphatase activity in the presence of chondroitin sulphate.[374]

Heparin.—The isolation of L-iduronic acid from an acid hydrolysate of the crystalline barium salt of heparin has been reported.[375] It was established that this acid was not derived from contamination by dermatan sulphate, nor by C-5 epimerisation of D-glucuronic acid. It was considered that the previous conditions employed for acid degradation were too drastic to give a completely representative spectrum of degradation products. Periodate oxidation of the sodium salt of heparin[376] resulted in destruction of the D-glucuronic acid residues. After borohydride reduction and acid hydrolysis, deamination with nitrous acid gave 2,5-anhydro-*aldehydo*-D-mannose-6-sulphate which was isolated as a brucinium salt. It was concluded that this placed the two sulphate groups (per tetrasaccharide unit) on the C-6 of the 2-amino-2-deoxy-D-glucose residues rather than on the D-glucuronic acid units. Other workers[377] have investigated the location of the sulphate esters by methylation analysis. Methylation was effected by dimethyl sulphate in aqueous alkali after replacement of the *N*-sulphate groups by *N*-acetyl substituents. Four extended methylations gave a material with 13% methoxy content which was theoretical for the observed three ester sulphate residues per tetrasaccharide. Analysis of the methylated monomers obtained from reduction of the carboxy-ester groups and

[371] T. Harada, K. Murata, T. Fujiwara, and T. Furuhashi, *Biochim. Biophys. Acta*, 1969, **177**, 676.
[372] I. Olsson, *Biochim. Biophys. Acta*, 1969, **177**, 241.
[373] N. Katsura and E. A. Davidson, *Biochim. Biophys. Acta*, 1969, **184**, 503.
[374] Y. Kawai, N. Seno, and K. Auno, *Analyt. Biochem.*, 1969, **32**, 314.
[375] M. L. Wolfrom, S. Honda, and P. Y. Wang, *Carbohydrate Res.*, 1969, **10**, 259.
[376] M. L. Wolfrom, P. Y. Wang, and S. Honda, *Carbohydrate Res.*, 1969, **11**, 179.
[377] I. Danishefsky, H. Steiner, A. Bella jun., and A. Friedlander, *J. Biol. Chem.*, 1969, **244**, 1741.

acid hydrolysis indicated that one-third of the D-glucuronic acid was sulphated at C-2 (the remainder being unsulphated), that most of the 2-amino-2-deoxy-D-glucose was sulphated at C-6 with some 3,6-disulphated, and that unsulphated residues were also present. The sulphate ester groups were apparently non-uniformly distributed.

Study of the nitrous acid deamination of the model disaccharide 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-glucuronic acid indicated that concomitant with the cleavage of the glycosidic linkage between the two units, epimerisation of the D-glucuronic acid residues to L-iduronic acid occurs.[378] The care needed in interpretation of deamination reactions during polysaccharide structural investigations was apparent.

The interaction of heparin with basic dyes has been studied and the observed visible spectra suggested to be consistent with those expected from 'accepted structure' of heparin.[379]

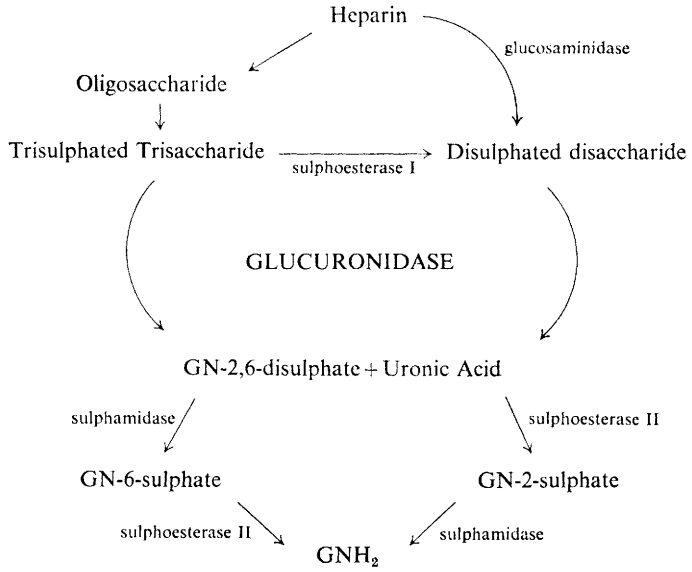
Further studies have been reported on the enzymic degradation of heparin by enzyme preparations from *Flavobacterium heparinum*.[380] Degradation to oligosaccharides appeared to occur before enzymic desulphation. Enzymes were isolated that allowed a complete scheme to be postulated for the degradation of heparin by this organism (Scheme 4).[381, 382] Other workers[383] have investigated the solubilisation of the carbohydrate sulphoamide sulphohydrolase and the carbohydrate sulphate sulphohydrolase of *F. heparinum*.

A heparin–protein complex isolated from ox liver capsule has been characterised.[384] β-Carbonyl elimination with or without catalytic hydrogenation followed by fractionation of the products indicated that the polysaccharide chain was covalently linked to the protein. Xylose (0·68%) was also reported in the preparation.

An undersulphated heparin-like polysaccharide in bovine milk was thought to be associated with a lipoprotein lipase.[385]

Hyaluronic Acid.—The separation of hyaluronic acid from whole sinovial fluid has been described.[386] Fractionation on agarose gels enabled the polydispersity and $\overline{DP}$ of the total hyaluronic acid to be determined. The application to the assessment of anti-inflammatory drugs was evaluated. Hyaluronic acid from rheumatoid sinovial fluid was associated with a protein that from immunochemical analyses appeared to be identical with human IgG.[387] Normal fluid did not show this association. Fractionated

[378] F. Yamaguchi, M. Kosaki, and Z. Yosizawa, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 721.
[379] T. F. Yen, M. Davar, and A. Rembaum, *Biochim. Biophys. Acta*, 1969, **184**, 646.
[380] C. P. Dietrich, *Biochemistry*, 1969, **8**, 2089.
[381] C. P. Dietrich, *Biochemistry*, 1969, **8**, 3342.
[382] C. P. Dietrich, *Biochem. J.*, 1969, **111**, 91.
[383] B. A. Law, A. G. Lloyd, G. Embery, and G. B. Wisdom, *Biochem. J.*, 1969, **115**, 10P.
[384] A. Serafini-Fracassini, J. J. Durward, and L. Floreani, *Biochem. J.*, 1969, **112**, 167.
[385] T. Olivecrona and U. Lindahl, *Acta Chem. Scand.*, 1969, **23**, 3587.
[386] M. J. How and V. J. W. Long, *Clinica. Chim. Acta*, 1969, **23**, 251.
[387] M. J. How, V. J. W. Long, and D. R. Stanworth, *Biochim. Biophys. Acta*, 1969, **194**, 81.



Scheme 4

hyaluronic acid from normal fluids contained 4·6% protein, whilst that from rheumatoid fluid contained 3·5—7·5% protein; the amino-acid composition showed no significant differences.

Hyaluronic acid isolated from bovine heart valves and purified by sedimentation equilibrium in a caesium chloride density gradient contained 15% protein.[388] Data obtained from light scattering, viscosity, and sedimentation measurements suggested that the hyaluronic acid was similar in size and configuration to that isolated from ox sinovial fluid.

Hyaluronic acid extracted from the mucoid layer of rooster combs with water at 4° had limiting viscosities as high as 13,000 ml g^{-1} and contained weak bonds that were irreversibly broken on heating at neutral pH above 65°.[389] Purified hyaluronic acid did not show these properties and protein did not appear to be a component of the structure formed by these bonds. It was therefore considered that the polysaccharide chains of hyaluronic acid in the connective tissue matrix were organised into a macromolecular network.

Tentative experiments involving alkaline degradation indicated a decrease in the threonine (68%) and serine (53%) contents of a hyaluronic acid preparation from vitreous humor.[390] Partial acid hydrolysis gave a glycopeptide containing arabinose and glucose which, by analogy with

[388] F. A. Meyer, B. N. Preston, and D. A. Lowther, *Biochem. J.*, 1969, **113**, 559.
[389] D. A. Swann, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 571.
[390] A. H. Wardi, W. S. Allen, D. L. Turner, and Z. Stary, *Biochim. Biophys. Acta*, 1969, **192**, 151.

chondroitin sulphate (xylose and galactose), suggested that arabinose was involved in the carbohydrate–peptide bond. Other workers[391] have reported the finding of an unidentified acidic fragment in a hydrolysate of vitreous humor hyaluronic acid.

Analysis of the products of hyaluronic acid degraded by ascorbic acid indicated that degradation proceeded by an essentially random cleavage of glycosidic bonds.[392] Determination of the relative proportion of terminal D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose residues indicated that the 2-acetamido-2-deoxy-D-glucosidic bond was more readily broken. Swan[393] also found no differences in the chemical identities of hyaluronic acid components after ascorbic acid degradation. Sedimentation, diffusion, and sedimentation equilibrium analyses gave values for $\overline{M}_w$ of $1{\cdot}2 \times 10^6$ and $6{\cdot}5 \times 10^4$ before and after degradation respectively. Since prolonged treatment did lower the molecular weight further, it was suggested that the initial material was a heterogeneous macromolecule containing hyaluronic acid or an aggregate.[393]

Further aspects of the biosynthesis of hyaluronic acid by *Streptococcus* have been elucidated.[394] Hyaluronic acid chain elongation occurred by transfer of monomer units from UDP derivatives to the non-reducing chain ends of endogenous oligosaccharides. These oligosaccharides could be released from the enzyme by acid treatment or by degradation with streptococcal hyaluronidase. The oligosaccharide released was chemically indistinguishable from hyaluronic acid.

Keratan Sulphate.—Acid hydrolysis of corneal keratan sulphate followed by electrophoresis gave, *inter alia*, 2-acetamido-1-(L-β-aspartamido)-1,2-dideoxy-β-D-glucose in a yield corresponding to 10% of the keratan sulphate bound aspartic acid.[395] This has established the identity of the carbohydrate–peptide linkage in this sulphated glycosaminoglycan. Proteolytic digestion of human knee-joint cartilage[396] gave keratan sulphate peptides with varying sulphate contents. The glycopeptides apparently contained mannose, xylose, and fucose in addition to the repeating unit components.

Biosynthesis of Mammalian Glycosaminoglycans.—The sulphation of chondroitin sulphate in embryonic chick cartilage by incorporation of [^{35}S]sulphate has been studied.[397] Microsomal and soluble enzyme preparations catalysed the transfer of [^{35}S]sulphate from adenosine-3′-phosphate-5′-sulphatophosphate into both chondroitin 4- and 6-sulphates.

[391] A. H. Wardi, W. S. Allen, G. A. Michos, and D. L. Turner, *Biochim. Biophys. Acta*, 1969, **184**, 474.
[392] R. L. Cleland, A. C. Stoolmiller, L. Roden, and T. C. Laurent, *Biochim. Biophys. Acta*, 1969, **192**, 385.
[393] D. A. Swann, *Biochem. J.*, 1969, **114**, 819.
[394] A. C. Stoolmiller and A. Dorfman, *J. Biol. Chem.*, 1969, **244**, 236.
[395] J. R. Baker, J. A. Ciffonelli, and L. Roden, *Biochem. J.*, 1969, **115**, 11P.
[396] H. Greiling and H. W. Stuhlsatz, *Z. physiol. Chem.*, 1969, **350**, 449.
[397] H. C. Robinson, *Biochem. J.*, 1969, **113**, 543.

However, since the pH, ionic strength, donor concentration, acceptor concentration, and incubation time all affected both the activity and the type of sulphation it was concluded that studies on the cell-free incorporation of sulphate into chondroitin sulphate were of questionable significance. A galactosyl transferase from embryonic chick cartilage catalysed the transfer of UDP-D-galactose-[^{14}C] to endogenous acceptor and exogenous substrates.[398] The structures of the products of reaction were typical of the linkage region in chondroitin sulphate. A D-glucuronosyl transferase, which catalysed the transfer of D-glucuronic acid from a UDP-D-glucuronic acid on to the non-reducing terminal D-galactose introduced by the galactose transferase, was also isolated.[399] A particulate enzyme preparation from chick embryo cartilage synthesised chondroitin sulphate (25%) and chondroitin (75%) from 3′-phosphoadenosine-5′-phosphosulphate, UDP-2-acetamido-2-deoxy-D-glucose, and UDP-D-glucuronic acid-[^{14}C].[400]

The effects of manganese on chondroitin sulphate synthesis in chick epiphyseal cartilage,[401] and of calcitonin on acidic glycosaminoglycan synthesis by embryo calf bone cells *in vitro*,[402] have been studied.

The glycosaminoglycans of cultivated connective tissue have been isolated and characterised to assess the effects of carrageenin and cortisone.[403] Hyaluronic acid, heparan sulphate, chondroitin 4- and 6-sulphates, an under-sulphated chondroitin sulphate, and dermatan sulphate were identified in cotton-wick granulation tissue.[403]

Induced effects on the [^{35}S]sulphate metabolism of acidic glycosaminoglycans of human skin have been studied with respect to both epidermal and dermal tissue.[404] The effect of vitamin A deficiency on the hexosamine-containing substances of healing wounds in guinea pigs [405] and the effect of vitamin A on the biosynthesis of sulphated glycosaminoglycans in cultured neoplastic mast cells [406] have been investigated.

An unusually high turnover rate of the keratan sulphate-like polysaccharide in granulation tissue has been observed.[407]

Polysaccharide Sulphates and Other Polysaccharides from Seaweeds

Odontalan, a sulphated polysaccharide of the red seaweed *Odonthalia corymbifera*, contained galactose and 6-*O*-methyl galactose as the principal

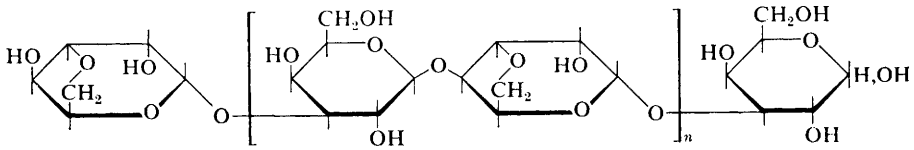
[398] T. Helting and L. Roden, *J. Biol. Chem.*, 1969, **244**, 2790.
[399] T. Helting and L. Roden, *J. Biol. Chem.*, 1969, **244**, 2799.
[400] J. E. Silbert and S. DeLuca, *J. Biol. Chem.*, 1969, **244**, 876.
[401] R. M. Leach jun., A. M. Muenster, and E. M. Wien, *Arch. Biochem. Biophys.*, 1969, **133**, 22.
[402] T. J. Martin, G. S. Harris, R. A. Merrick, and J. R. E. Fraser, *Experientia*, 1969, **25**, 375.
[403] E. V. Chandrasekaran and B. K. Backhawat, *Biochim. Biophys. Acta*, 1969, **177**, 265.
[404] S. A. Barker and J. F. Kennedy, *Carbohydrate Res.*, 1969, **11**, 27.
[405] C. J. Bates, C. I. Levene, and E. Kodicek, *Biochem. J.*, 1969, **113**, 783.
[406] D. B. Thomas and C. A. Pasternak, *Biochem. J.*, 1969, **111**, 407.
[407] K. Ivaska, E. Heikkinen, A. Lehtonen, and E. Kulonen, *Acta Chem. Scand.*, 1969, **23**, 3615.

components.[408] The mass spectra of the di-*O*-isopropylidene derivatives were employed for identification purposes. Using the Smith degradation technique (after removal of the sulphate groups) it was demonstrated that (1 → 3)- and (1 → 4)-linked galactose units were present in the λ-polysaccharide of *Tichocarpus crinitus*.[409]

The sulphated polysaccharide from the red seaweed *Phyllymenia cornea*, phyllymenan, contained a broader distribution of methylated galactose units.[410] Acid hydrolysis gave D-galactose, 2-*O*-methyl-D-galactose, 4-*O*-methyl-L-galactose, 6-*O*-methyl-galactose, and xylose. Two oligosaccharides were characterised in a partial acid hydrolysate, 4-*O*-β-D-galactopyranosyl-D-galactose and 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-D-galactose. All the sulphate substituents were alkali stable. Fucoidin, laminarin, alginic acid, and a sulphated glucuronoxylofucan have been isolated from the brown seaweed *Fucus vesiculosus*.[411]

The water-soluble sulphated polysaccharide of *Cladophora rupestris* has been investigated by periodate oxidation.[412] Application of three successive Smith degradations confirmed the highly branched nature of the polysaccharide and the presence of 1,4-linked xylose units. It was concluded that the galactofuranose units were substituted at C-6 either by sulphate or by another sugar residue.

Further studies have been reported on the application of a bacterial agarase to the degradation of agarose, porphyran, and alkali-treated porphyran. An extracellular agarase was obtained from cultures of *Cytophaga* (NCMB 1327) which, in contrast to previous ultrasonic extracts, did not contain contaminating *exo*-agarase activity or sulphatase activity.[413] Degradation of agarose with the *endo*-agarase gave a series of neoagarosaccharides (58) which were separated by gel permeation chromatography.[414] Alkali-treated porphyran gave a similar series of oligosaccharides together with others containing 6-*O*-methyl-D-galactose. It was



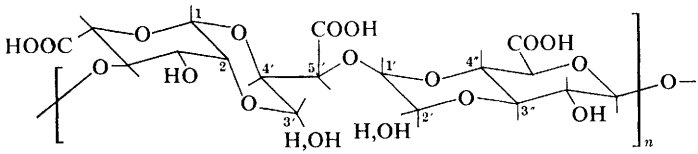
$n = 1, 2, 3$

(58)

[408] A. I. Usov and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1968, **38**, 234.
[409] A. I. Usov, M. A. Rekhter, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1969, **39**, 905.
[410] J. R. Nunn and H. Parolis, *Carbohydrate Res.*, 1969, **9**, 265.
[411] E. J. Bourne, P. Brush, and E. Percival, *Carbohydrate Res.*, 1969, **9**, 415.
[412] P. G. Johnson and E. Percival, *J. Chem. Soc.* (*C*), 1969, 906.
[413] M. Duckworth and J. R. Turvey, *Biochem. J.*, 1969, **113**, 139.
[414] M. Duckworth and J. R. Turvey, *Biochem. J.*, 1969, **113**, 687.

apparent that the enzyme cleaved at the D-galactose residues at about four to five times the rate at the 6-*O*-methyl-D-galactose residues. Porphyran itself gave smaller yields of the same oligosaccharides and a large amount of undegraded polymeric material. It was concluded therefore that the 6-*O*-methyl groups were distributed randomly on 50% of the D-galactose units but that the 6-sulphate groups on the L-galactose units tended to occur in blocks. A more detailed study of the action pattern of the enzyme has been made.[415]

The abnormally low periodate oxidation limit of alginate (0·45—0·55 mole of oxidant per 'anhydro-hexurono' unit) has been explained in terms of intramolecular hemiacetal formation.[416] Reduction of the periodate-oxidised alginate with sodium borohydride followed by reoxidation gave a total oxidant consumption of 0·95 moles per mole of 'anhydro-hexurono' unit which was increased to the theoretical value (1·0) on repetition of the sequence. If random periodate oxidation was assumed, and two neighbouring units were assumed not to be simultaneously oxidised, then intramolecular hemiacetal formation as in (59) would lead to values of



(59)

0·48 and 0·95 for the first and second periodate oxidation limits. These findings further emphasise the care needed in the interpretation of periodate oxidation data.

The role of intramolecular autocatalysis in the acid hydrolysis of polysaccharides containing 1,4-linked hexuronic acid units has been evaluated.[417] At pH 2—4 oligomannuronic acids (OM) were hydrolysed at 100° more quickly than oligoguluronic acids (OG). Mixed oligouronides were hydrolysed at intermediate rates and a good correlation could be obtained between the observed rate and that calculated from k_{OM}, k_{OG}, and the M/G ratio. After partial hydrolysis of a mixed oligouronide 80% of the isolated dimer fragments contained both monomers in the same molecule and of these 90% had the mannuronic acid residue as the non-reducing terminal unit. These results supported the view that the high rate of hydrolysis of 1,4-polyuronides in weakly acidic media was due largely to intramolecular catalysis of glycosidic cleavage by the carboxy-group in the respective aglycone units.

[415] M. Duckworth and J. R. Turvey, *Biochem. J.*, 1969, **113**, 693.
[416] B. Larsen and T. J. Painter, *Carbohydrate Res.*, 1969, **10**, 186.
[417] O. Smidsrod, B. Larsen, T. J. Painter, and A. Haug, *Acta Chem. Scand.*, 1969, **23**, 1573.

Comparison of the results of a theoretical treatment with experimentally observed results with a partially degraded alginate indicated that the structure of the alginate molecule closely resembled that of a copolymer formed according to the 'penultimate-unit' theory of addition copolymerisation.[418] The development of compositional heterogeneity in alginate degraded in homogeneous solution has been investigated.[419] In three random depolymerisation systems liberation of discrete polymeric fragments of different composition was indicated. It was concluded that the observed behaviour was not due to the presence of 'weak linkages' between differently composed segments of chains, but due to a typical random depolymerisation of a block copolymer. It was suggested that this approach could be of general value as a test of blockwise arrangement of monomer units in a polysaccharide.

An enzyme system from *Azotobacter vineandii* epimerised D-mannuronic acid residues to L-guluronic acid in a polymer chain with little or no concomitant depolymerisation.[420] Enzyme-modified alginate had a similar block structure to ordinary algal alginate of the same composition.

[418] B. Larsen, T. J. Painter, A. Haug, and O. Smidsrod, *Acta Chem. Scand.*, 1969, **23**, 355.
[419] A. Haug, B. Larsen, O. Smidsrod, and T. J. Painter, *Acta Chem. Scand.*, 1969, **23**, 2955.
[420] A. Haug and B. Larsen, *Biochim. Biophys. Acta*, 1969, **192**, 557.

7

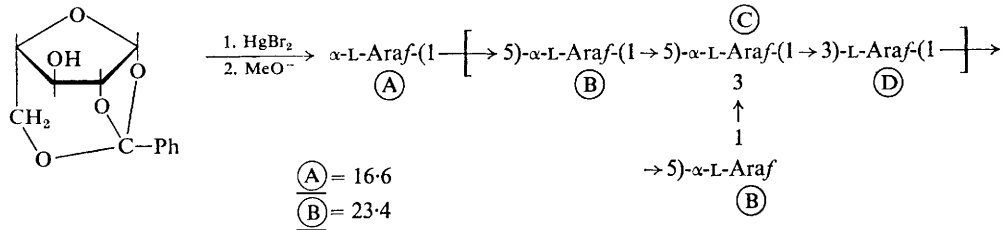
Chemical Synthesis and Modification of Polysaccharides

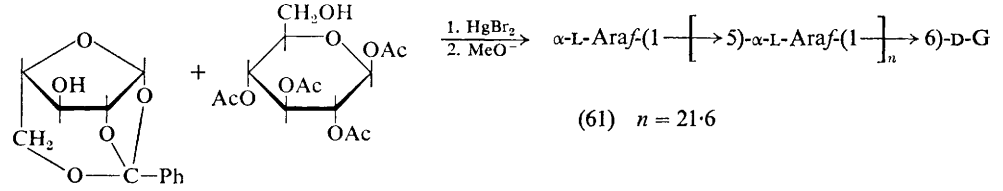
The synthesis of a branched arabinan reported previously has been described in more detail.[421] Polymerisation of the tricyclic β-L-arabinofuranose-1,2,5-orthobenzoate gave a 50% yield of a branched α-L-arabinan. Methylation, periodate oxidation, and hydrolysis investigations led to a picture of the general features of the polysaccharide as shown in (60). Polymerisation of 3-*O*-acetyl-β-L-arabinofuranose 1,2,5-orthobenzoate with 1,2,3,4-tetra-*O*-acetyl-β-D-glucose as initiator gave an essentially linear (1 → 5)-α-L-arabinan terminated at the reducing end by a D-glucose residue (61) (Scheme 5). This polymer contained a small number of (1 → 2) linkages, presumably owing to formation of the alternative carbonium ion (62) as shown in Scheme 6.

The polymerisation of the trimeric macrocyclic orthoacetate of 4,6-*O*-benzylidene-α-D-glucopyranose gave a mixture of saccharides of broad molecular weight distribution (Scheme 7).[422] Isolation of the high-molecular-weight fraction and subsequent periodate oxidation indicated the formation of an essentially β-(1 → 3)-D-glucan of DP 30 (63). This material closely resembled the G chain of laminarin.

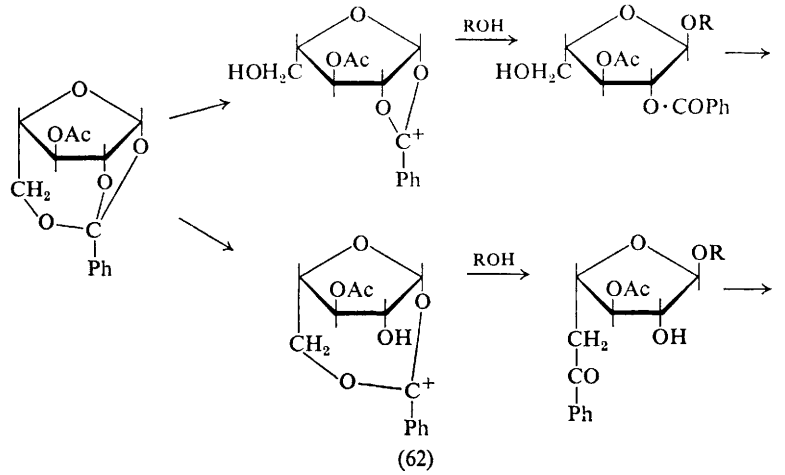
Phosphorus pentafluoride-initiated polymerisation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-α-D-mannopyranose at −78 °C in dichloromethane, followed by debenzylation gave a polymer, $\overline{DP}_n$ up to 1050.[423] Polymerisation of the analogous triacetate gave a less stereoregular product. Periodate consumption and concomitant formic acid production were theoretical for a poly-α-(1 → 6′)-anhydro-D-mannose. A similar method of polymerisation gave a stereoregular polymer from 1,6-anhydro-2,3,4-tri-*O*-benzyl-α-D-glucopyranose.[424] The loss of stereoregularity appeared to result from the use of conditions which converted the propagating site from a trialkyloxonium ion into a glycosylcarbonium ion. In the esterified monomer the glycosylcarbonium ion would be stabilised by C-2 ester participation.

[421] N. K. Kochetkov, A. F. Bochkov, and G. Yazlovetsky, *Carbohydrate Res.*, 1969, **9**, 49.
[422] N. K. Kochetkov and A. F. Bochkov, *Carbohydrate Res.*, 1969, **9**, 61.
[423] J. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, 1969, **91**, 1161.
[424] J. Zachoval and C. Schuerch, *J. Amer. Chem. Soc.*, 1969, **91**, 1165.

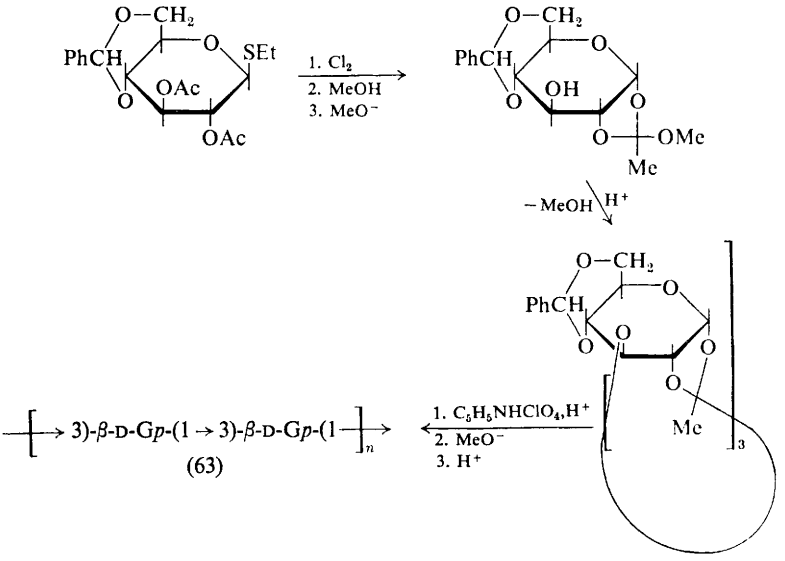

1. HgBr2
2. MeO−
α-L-Araf-(1→5)-α-L-Araf-(1→5)-α-L-Araf-(1→3)-L-Araf-(1→
A
B
C
D
3
1
→5)-α-L-Araf
B
A = 16·6
B = 23·4
C = 15·6
D = 3·8
(60)


1. HgBr2
2. MeO−
α-L-Araf-(1→5)-α-L-Araf-(1→6)-D-G
n
(61) n = 21·6

Scheme 5



Scheme 6



Scheme 7

Only relatively low-molecular-weight oligomers were formed when 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose was fused with toluene-*p*-sulphonic acid or zinc chloride.[425] Gentiodextrins of up to $\overline{DP}_n$ 9—10 were isolated. Oligomers containing (1 → 2), (1 → 3), or (1 → 4) linkages were

[425] D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohydrate Res.*, 1969, **11**, 453.

obtained from the appropriate tetra-acetates. Use of toluene-*p*-sulphonic acid as catalyst gave stereoregular products, whereas zinc chloride-catalysed polymerisation gave products containing both α- and β-linkages.[426]

The alkaline saponification of cellulose toluene-*p*-sulphonates gave polysaccharides containing 2,3- or 3,6-anhydro-sugar units which could be allowed to react further to give a series of heteroglucan polymers.[427] By this technique polysaccharides containing D-mannose, D-altrose, 2-amino-2-deoxy-D-glucose, or 2-amino-2-deoxy-altrose in addition to glucose were obtained.

Dextrinisation of amylopectin with D-xylose resulted in the formation of a highly branched codextrin containing 13·4% D-xylose.[428] The D-xylose was primarily introduced as non-reducing termini together with some 1,4- and 1,2,4-linked units.

Polysaccharide phosphates containing phosphate monoesters with a D.S. of up to 1·75 have been prepared by the reaction of the polysaccharide with tetrapolyphosphoric acid–trialkylamine mixture.[429] Phosphate esters introduced into amylose, amylopectin, waxy corn starch cellulose, and dextran were stable to saponification but less resistant to acid hydrolysis.

The alkaline-degraded dextran used to prepare iron–dextran complexes has been shown to contain on average 1 mole of glucometasaccharinic acid per chain of dextran.[430] D-Gluconic acid end-groups have been confirmed in unbleached cotton cellulose and the problems associated with its analysis discussed.[431]

Structural studies of partially methylated cotton cellulose suggested that the elementary fibril is the basic structural element and had a surface which methylated rapidly.[432] The distribution of hydroxyethyl substituents in hydroxyethyl starch [433, 434] and dextran [433] have been determined. The importance of inclusion of the 1,2-*O*-ethylene-α-D-glucofuran and pyranose formed in the hydrolysate has been demonstrated.[433] The distribution of substituents in *O*-(2-aminoethyl)-cellulose has been determined after conversion by diazotisation to the hydroxy-derivative.[435]

Persilylation of cellulose, amylose, and poly(vinyl alcohol) by reaction with *N*-trimethylsilylacetamide has been reported.[436] The preparation and

[426] D. McGrath, E. E. Lee, and P. S. O'Colla, *Carbohydrate Res.*, 1969, **11**, 461.
[427] L. S. Gal'braikh, Z. A. Rogovin, M. K. Belyakova, and S. I. Polukhina, *Makromol. Chem.*, 1969, **122**, 38; S. I. Polukhina, L. S. Gal'braikh, and Z. A. Rogovin, *Vysokomol. Soedineniya*, 1968, **10**, *A*, 2039.
[428] Y. Ghali, B. A. Lewis, and F. Smith, *Cereal Chem.*, 1968, **45**, 589.
[429] R. L. Whistler and G. A. Towle, *Arch. Biochem. Biophys.*, 1969, **135**, 396.
[430] I. Bremner, J. S. G. Cox, and G. F. Moss, *Carbohydrate Res.*, 1969, **11**, 77.
[431] K. Larsson and O. Samuelson, *Carbohydrate Res.*, 1969, **11**, 144.
[432] S. Haworth, D. M. Jones, J. G. Roberts, and B. F. Sager, *Carbohydrate Res.*, 1969, **10**, 1.
[433] A. N. DeBelder and B. Norrman, *Carbohydrate Res.*, 1969, **10**, 391.
[434] G. N. Bollenback, R. S. Golik, and F. W. Parish, *Cereal Chem.*, 1969, **46**, 304.
[435] J. E. Roberts and S. P. Rowland, *Canad. J. Chem.*, 1969, **47**, 1571.
[436] K. Bredereck, K. Strunk, and H. Menrad, *Makromol. Chem.*, 1969, **126**, 139.

properties of carboxymethylated cellulose have been described[437] and the fractional precipitation studied.[438] The photochemical degradation of cellulose[439] and the thermal degradation of 2-*O*-methyl cellulose[440] have been studied. The factors affecting the selective acetolysis of cellulose to material of low DP have been evaluated.[441]

[437] H. Vink, *Makromol. Chem.*, 1969, **122**, 134.
[438] M. Nakagaki, H. Sumada, A. Kondo, and J. Terrao, *J. Pharm. Soc. Japan*, 1969, **89**, 139.
[439] R. L. Desai and J. A. Shields, *Makromol. Chem.*, 1969, **122**, 134.
[440] P. C. Wollwage and P. A. Seib, *Carbohydrate Res.*, 1969, **10**, 589.
[441] H. M. Kaustinen, O. A. Kaustinen, and H. A. Swenson, *Carbohydrate Res.*, 1969, **11**, 267.

8
Physicochemical Properties

Further work on the conformational analysis of polysaccharides has been reported. Computerised model building with the aid of helical parameters has been used to investigate the conformation of alternating copolymers of the agar–carrageenan–chondroitin type.[442] Comparisons were made with the available crystal structure data on disaccharide repeat units. The geometrical properties of all allowed conformations were calculated and compared, and the ranges observed showed a remarkable similarity within the entire group of seaweed and animal polysaccharides of this type. Steric restrictions caused extended conformations with the main differences reflected in the chain flexibility as indicated by the proportion of allowed conformations. This tended to be lower for the acidic glycosaminoglycans due to the larger equatorial groups adjacent to each glycosidic linkage. *X*-Ray diffraction studies provided evidence for double helices in κ- and *i*-carrageenans.[443] Double helices with three disaccharide repeat units per complete turn of each single chain were indicated with the second chain in *i*-carrageenan displaced exactly half a pitch from the first chain. The significance of different linkage positions in relation to conformational analysis has been discussed for β-linked polysaccharides.[444]

A helical structure has also been demonstrated in the β-(1 → 3)-xylan from cell walls of *Penicillus dumetosus*.[445] This structure contained, however, three intertwined xylan chains to form a three-stranded helix. Each strand of the helix contained six xylose residues per turn with a pitch of *ca.* 18 Å. A novel type of interchain bonding was proposed, in which a triad of cyclic hydrogen bonds was formed between oxygen atoms, one from each individual chain.

An improved method has been reported for the fractionation of maize and amylo-maize starches by complex formation from an aqueous dispersion after pretreatment with DMSO.[446] Examination of the properties of the fractionated materials confirmed the view that the abnormality of amylo-maize starches was entirely due to the presence of short-chain

[442] D. A. Rees, *J. Chem. Soc.* (*B*), 1969, 217.
[443] N. S. Anderson, J. W. Cambell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 1969, **45**, 85.
[444] D. A. Rees and W. E. Scott, *Chem. Comm.*, 1969, 1037.
[445] E. D. T. Atkins, K. D. Parker, and R. D. Preston, *Proc. Roy. Soc.*, 1969, *B*, **173**, 209.
[446] G. K. Adkins and C. T. Greenwood, *Carbohydrate Res.*, 1969, **11**, 217.

amylosic material. True scattering intensities have been obtained from amylose and amylopectin by degradation with α-amylase in the light-scattering cell.[447] This technique allowed the considerable contribution from dust to be allowed for. The effects of solvent on the formation of the starch–iodine complex have been examined.[448] The results were interpreted in terms of the water required for the rigid helix formation in the complex. The loose coil in solution could be maintained by association with other solvents. Other workers [449] have investigated the nature of the reaction of iodine with a number of polysaccharides. Only a xylan showed similar complex formation to that observed with amylose.

The o.r.d. spectra of amylose films have been investigated but conformational information could not be obtained from these data alone.[450] The melting and glass transition studies of amylose esters [451] and the melting temperature of cellulose tricarbanilate [452] have been determined.

It has been concluded that the partial free draining effect on intrinsic viscosity, sedimentation, and diffusion constant for cellulose nitrate solutions could not be ignored.[453] The solvation of cellulose has been investigated in DMF and dimethylacetamide to which either dinitrogen tetroxide or nitrosyl chloride had been added.[454] Cellulose was recovered after contact with water or lower alcohols in an apparently undegraded form.

The degradation of both 'V' amylose hydrate and a hydrated amorphous amylose to glucose and maltose by gaseous hydrogen chloride has been investigated.[455]

N.m.r. and o.r.d. measurements have been reported for *N*-acetylneuraminic acid and colominic acid and their changes on formation of the intramolecular lactone in the case of colominic acid noted.[456] Study of the o.r.d. spectrum and viscosity behaviour of polygalacturonic acid in solution suggested a model in which the flexibility and extension of the molecule is related to the repulsion of adjacent charged carboxy-groups.[457]

The determination of DP of a seaweed xylan has been determined using nitrated xylan [458] and extrapolation of the osmotic pressure data.

Physicochemical measurements on dextran and dextran tricarbanilate indicated that the carbanilation reaction was polymer-analogous up to

[447] W. Banks, C. T. Greenwood, and J. Sloss, *Carbohydrate Res.*, 1969, **11**, 399.
[448] W. T. Smith jun. and G. T. Smith, *Carbohydrate Res.*, 1969, **10**, 598.
[449] B. D. E. Gaillard, N. S. Thompson, and A. J. Morak, *Carbohydrate Res.*, 1969, **11**, 509.
[450] R. M. Purvinas and H. F. Zobel, *Carbohydrate Res.*, 1969, **10**, 129.
[451] J. M. G. Cowie, P. M. Toporowski, and F. Costaschuk, *Makromol. Chem.*, 1969, **121**, 51.
[452] R. S. Colborne, *Makromol. Chem.*, 1969, **128**, 197.
[453] K. Kamide, *Makromol. Chem.*, 1969, **122**, 261.
[454] R. G. Schweiger, *Chem. and Ind.*, 1969, 296,
[455] R. P. Panzica, G. U. Yuen, and B. Zaslow, *Carbohydrate Res.*, 1969, **10**, 343.
[456] A. Kimura, K. Tsurumi, T. Fukushima, *J. Med. Sci.*, 1969, **15**, 55.
[457] R. W. Stoddart, I. P. C. Spires, and K. F. Tipton, *Biochem. J.*, 1969, **114**, 863.
[458] W. Mackie, *Carbohydrate Res.*, 1969, **9**, 247.

DP 150,000.[459] Some evidence of aggregation of dextran molecules was obtained. The folded-chain structure of cellulose has been refuted [460] and the structure of chitin or parallel chain systems investigated.[461]

[459] W. Burchard and B. Pfannemuller, *Makromol. Chem.*, 1969, **121**, 18.
[460] R. E. Mark, *Science*, 1969, **164**, 72.
[461] J. Blackwell, *Biopolymers*, 1969, 7, 287.

9
Hydrolytic Enzymes

An n.m.r. study of the anomeric species produced by D-glucosidases from some D-glucosides and D-glucans in D_2O indicated that *endo*-enzymes hydrolysed with retention of the anomer configuration whilst *exo*-enzymes degraded with inversion.[462] Studies by g.l.c. showed that pancreatic α-amylase liberated maltose and maltotriose in the α-form from amylose, whilst β-amylase from barley gave the β-anomeric form of maltose.[463] Extension of this technique to a lactase and sucrase showed that β-D-galactose was obtained by the action of lactase on lactose and α-D-glucose from sucrose by the action of sucrase.

A method for the determination of the action pattern of glycanases based on measurement of the average chain length of the reaction products with time of reaction has been described.[464]

Methyl terminal 4-deoxy, terminal 4-*O*-ethyl, and terminal 4-*O*-butyl-maltodextrins have been synthesised *via* perbenzylated methyl terminal 4-hydroxy-maltodextrins.[465] α-Amylase degraded mixed methyl terminal 4-deoxy-maltodextrins to maltose, 4-*O*-α-(4-deoxy-D-*xylo*-hexopyranosyl)-D-glucose, methyl maltoside and methyl-D-glucopyranoside.

Factors contributing to the catalysis by porcine pancreatic α-amylase have been investigated.[466] Taka amylase A degraded phenyl α-maltosides in which the hydroxy-groups of the 'reducing' terminal glucose residue were monomethylated in any position but only that in which the C-2 hydroxy-group was substituted in the non-reducing glucose residue.[467] The kinetics and mode of action of hydrolysis and transfer reactions of saccharifying α-amylase from *B. subtilis* have been investigated.[468]

'Stubbed' oligosaccharides prepared by acid hydrolysis of waxy maize starch followed by degradation with γ-amylase have been used to investigate

[462] D. E. Everleigh and A. S. Perlin, *Carbohydrate Res.*, 1969, **10**, 87.
[463] G. Semenza, H. Ch. Curtius, O. Raunhardt, P. Hore, and M. Muller, *Carbohydrate Res.*, 1969, **10**, 417.
[464] K. K. Tung and J. H. Nordin, *Analyt. Biochem.*, 1969, **29**, 84.
[465] R. E. Wing and J. N. BeMiller, *Carbohydrate Res.*, 1969, **10**, 371.
[466] J. Wakim, M. Robinson, and J. A. Thoma, *Carbohydrate Res.*, 1969, **10**, 487.
[467] M. Isemura, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1969, **66**, 77.
[468] H. Yoshida, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1969 **66**, 183.
H. Yoshida, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1969, **65**, 741.
A. Yutani, K. Yutani, and T. Isemura, *J. Biochem. (Japan)*, 1969, **65**, 201.

the mode of action of α-amylase.[469] 'Stubbed' tri- and tetra-saccharides containing only single D-glucosyl side-chains were not hydrolysed, whilst higher stubbed oligosaccharides with single D-glucosyl side-chains were converted to tetrasaccharides, glucose and/or maltose. Extracts of sweet corn gave a separable mixture of a typical R-enzyme (acting only on amylopectin) and an isoamylase (acting on amylopectin and glycogen).[470] Isoamylase degradation of phytoglycogen gave a mixture of maltodextrins and a residual polysaccharide of glycogen rather than amylopectin-type structure. It was concluded that *in vivo* amylopectin was not therefore formed by enzymic debranching of a glycogen precursor. An α-D-glucosidase has been separated from D-enzyme in tomato and carrot extracts.[471]

Further enzymes, β- and γ-amylase, have been coupled, with retention of activity, to diazotised 3-(*p*-aminophenoxy)-2-hydroxypropyl ethers of cellulose.[472] Only β-amylase retained activity on coupling to 2-hydroxy-3-(*p*-isothiocyanatophenoxy)propyl ethers of cellulose. Both modes of coupling gave products with increased enzyme heat stability.

The mechanism of action of pullulanase has received attention from two groups of workers with conflicting results. Measurement of maltotriose production from pullulan by g.l.c. showed a linear increase in concentration with time of pullulanase incubation, indicative of an *exo* mode of action.[473] Other workers[474] suggested an *endo*-enzyme reaction on the basis of the degradation products. A series of oligosaccharides was produced with DP's of 3, 6, 9 . . ., 21, and the maltotriose production always lagged behind the extent of degradation of the pullulan.

An *endo*-α-D-(1 → 3) glucanase has been isolated and characterised from *Trichoderma viride*.[475] This enzyme appeared to be specific for α-(1 → 3) linkages, and was therefore considered of importance for structural investigations on fungal α-D-glucans. A β-(1 → 3)-D-glucanase from the yeast *Hanseniaspora valbyensis* appeared to be specific for β-(1 → 3)-linked units and to require at least three consecutive such units for hydrolysis to occur.[476] *exo*-β-(1 → 3)-Glucanases have been isolated and characterised from sea urchins,[477] the euglenoid flagellate *Euglena gracilis*,[478] and *Basidiomycetes* species QM 806.[479]

[469] K. Kainuma and D. French, *F.E.B.S. Letters*, 1969, **5**, 257.
[470] D. J. Manners and K. L. Rowe, *Carbohydrate Res.*, 1969, **9**, 107.
[471] D. J. Manners and K. L. Rowe, *Carbohydrate Res.*, 1969, **9**, 441.
[472] S. A. Barker, P. J. Somers, and R. Epton, *Carbohydrate Res.*, 1969, **9**, 257.
[473] K. Wallenfels, I. R. Rached, and F. Huche, *European J. Biochem.*, 1969, **7**, 231.
[474] G. S. Drummond, E. E. Smith, W. J. Whelan, and H. Tai, *F.E.B.S. Letters*, 1969, **5**, 85.
[475] S. Hasegawa, J. H. Nordin, and S. Kirkwood, *J. Biol. Chem.*, 1969, **244**, 5460.
[476] A. T. H. Abdelal and H. J. Phaff, *Canad. J. Microbiol.*, 1969, **15**, 697.
[477] A. V. Muchmore, D. Epel, A. M. Weaver, and R. T. Schimke, *Biochim. Biophys. Acta*, 1969, **178**, 551.
[478] D. R. Barras and B. A. Stone, *Biochim. Biophys. Acta*, 1969, **191**, 329, 342.
[479] T. E. Nelson, J. Johnson jun., E. Jantzen, and S. Kirkwood, *J. Biol. Chem.*, 1969, **244**, 5972.

The isolation of cellulases and carboxymethylcellulases from a variety of sources has been reported including *Polyporus schweinitzii*,[480] *Sterum sanguinolentum*,[481] *Chrysosporium lignorum*,[482] *Penicillium notatum*,[483] *Verticillium albo-atrum*,[484] and *Fusarium solani*.[485] A partial separation of the β-(1 → 6)-glucanase activity of an enzyme preparation from *Schizophyllum commune* from other activities has been reported.[486]

The inhibition of β-D-galactosidase activity by 1,2-dideoxy-D-*lyxo*-hex-1-enopyranose has been observed and attributed to a planar half-chair conformation.[487] α-D-Mannosidases, β-D-glucosidases, and 2-acetamido-2-deoxy-β-D-glucosidases were not inhibited. The purification and substrate specificities of α-D-galactosidases from sweet almonds,[488] *Vicia faba*,[489] and *A. niger* have been described.[490]

The α-D-mannosidase of *Arthrobacter* GJM-1 has been characterised with respect to its action on yeast mannans.[491] The enzyme showed an *exo*-pattern of activity and removed α-(1 → 2)- and α-(1 → 3)-linked side-chains from a *S. cerivisiae* mannan leaving a resistant predominantly α-(1 → 6)-linked core mannan.

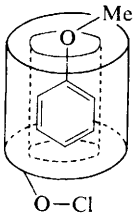
The action pattern of α-L-arabinofuranosidases from *A. niger*[492] and *Sclerotinia fructigena*[493] has been established.

[480] P. J. Bailey, W. Liese, R. Roesch, G. Keilicher, and E. G. Afting, *Biochim. Biophys. Acta*, 1969, **185**, 381.
[481] K. E. Eriksson and W. Rsedowski, *Arch. Biochem. Biophys.*, 1969, **129**, 689.
[482] B. Bucht and K. E. Eriksson, *Arch. Biochem. Biophys.*, 1969, **129**, 416.
[483] G. Petterson, *Arch. Biochem. Biophys.*, 1969, **130**, 286.
[484] P. Whitney, J. M. Chapman, and J. B. Heale, *J. Gen. Microbiol.*, 1969, **56**, 215.
[485] T. M. Wood, *Biochem. J.*, 1969, **115**, 457.
[486] J. G. H. Wessels, *Biochim. Biophys. Acta*, 1969, **178**, 191.
[487] Y. C. Lee, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 161.
[488] P. M. Dey, *Biochim. Biophys. Acta*, 1969, **191**, 644.
[489] P. M. Dey and J. B. Pridham, *Biochem. J.*, 1969, **115**, 47.
[490] O. P. Bahl and K. M. L. Agrawal, *J. Biol. Chem.*, 1969, **244**, 2970.
[491] G. H. Jones and C. E. Ballou, *J. Biol. Chem.*, 1969, **244**, 1043, 1052.
[492] A. Kaji, K. Tagawa, and T. Ichimi, *Biochim. Biophys. Acta*, 1969, **171**, 186.
[493] A. H. Fielding and R. J. W. Byrde, *J. Gen. Microbiol.*, 1969, **58**, 73.

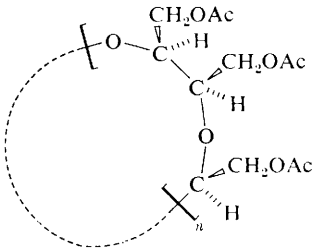
10
Miscellaneous

Selective aromatic substitution of anisole has been observed in the presence of cyclohexa-amylose (α-cyclodextrin). Chlorination with hypochlorous acid proceeded almost exclusively with *para*-orientation.[494] The observed results indicated that the effect was not entirely steric in character and participation of an intramolecular complexed hypochlorite residue on a rim hydroxy-group was suggested (64).

Periodate oxidation followed by borohydride reduction has been employed to generate large macrocylic rings from cyclodextrins.[495] The product obtained from cyclohepta-amylose was acetylated, and shown to be a cyclic



(64)



(65) $n = 7$

(66) $n = 6$

acetal with 14 oxygen and 21 carbon atoms, in a 35-membered macrocyclic system. The product was optically inactive and n.m.r. data were in accordance with the expected structure (65). An analogous product was obtained from a similar reaction sequence applied to cyclohexa-amylose (66).

The effect of polysaccharides in immune reactions has been extensively investigated by Hellsing.[496] From the results obtained it was concluded that the increased precipitation of antibodies by antigens in the presence of antigenically inactive polysaccharide was due to a steric exclusion mechanism.

[494] R. Breslow and P. Cambell, *J. Amer. Chem. Soc.*, 1969, **91**, 3085.
[495] J. W. Stoddart, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1969, **47**, 3213.
[496] K. Hellsing, *Biochem. J.*, 1969, **114**, 141, 145, 151, 475.

Diazotised *m*-aminobenzyloxymethyl cellulose has been used as a matrix for support of an immunosorbent to enable purification of antigens and antibodies.[497] Aminoethyl cellulose gave a stable and fully reversible immunosorbent after activation with cyanogen bromide.

Cellulose supports have been used for the sequential analysis of polyribonucleotides.[498]

4-*O*-(Tetra-*O*-acyl-β-D-mannopyranosyl)-D-erythritol has been characterised in a glycolipid from *Ustilago maydis* (corn smut fungus).[499]

Four sialo-glycosphingolipids with oligosaccharide chains of varying length have been isolated from gonads of the sea urchin *Strongylocentrotus intermedius*.[500] Both *N*-acetyl- and *N*-glycollyl-neuraminic acids were identified. Some glucoside residues in sialoglycolipids from *Echinodermata* have been shown to be in the furanose form.[501]

[497] R. V. Davies, R. M. Blanken, and K. J. Beagle, *Biochemistry*, 1969, **8**, 2706.
T. Kristiansen, L. Sundberg, and J. Porath, *Biochim. Biophys. Acta*, 1969, **184**, 93.
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[499] A. L. Fluharty and J. S. O'Brien, *Biochemistry*, 1969, **8**, 2627.
[500] N. K. Kochetkov, I. G. Zhukova, and G. P. Smirnova, *Doklady Akad. Nauk S.S.S.R.*, 1968, **180**, 996.
[501] I. G. Zhucova and G. P. Smirnova, *Carbohydrate Res.*, 1969, **9**, 366.

Index of Trivially-named Substances

Note: This index does not include those substances with the ending '-mycin' or '-micin', which can be found by reference to Chapter 20 in each Volume. Because each volume is cross-referenced generally only one entry per volume is given.

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