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Carbohydrate Chemistry

Volume 1

A Review of the Literature Published during 1967

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Organic formulae composed by Wright's Symbolset method

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Preface

It has been our aim in preparing this first Report to outline and correlate the majority of papers published in the field of carbohydrate chemistry during 1967. The terms of reference taken for Parts I and II are set out in the respective Introductions and emphasise the chemical rather than the biochemical aspects of the subject; to this extent therefore the coverage is selective. We would point out that the nucleoside section in particular is less than complete. We hope, however, to have produced a review from which the development of carbohydrate chemistry during the year can be followed in some detail.

Work available to the authors up to mid-January 1968 has been included. Papers which appeared in 1967 journals and which became available after that date will be covered in the Report for 1968, and the Reviewers will be glad to consider any 1967 papers which authors feel should have been included in this first issue. Research described in *Abstracts of the American Chemical Society Meetings* or *Diss. Abs.* was not abstracted.

Comments and advice which will enable subsequent Reports to be brought into line with the precise requirements of research chemists are invited.

We thank Dr. E. Mukmenev for help with the translation of Russian papers.

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

April 1968.

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Part II Macromolecules

Abbreviations

The following abbreviations have been used

DCC	dicyclohexylcarbodiimide
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
e.s.r.	electron-spin resonance
g.l.c.	gas–liquid chromatography
i.r.	infrared
LAH	lithium aluminium hydride
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
THF	tetrahydrofuran
t.l.c.	thin-layer chromatography
TMS	trimethylsilyl

Part I

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

1
Introduction

In this Report we have attempted to correlate all aspects of the organic chemistry of the mono-, di-, and tri-saccharides described in papers published during 1967. Biochemical aspects, such as biosynthesis, metabolism, enzymology, and physiological activity, have not generally been covered. We trust that those who publish in fringe areas will be understanding if they find their work unreported; we shall rectify any important omissions in the next issue if they are brought to our attention.

The adopted method of classification is based upon compound groups, and reports on chemical reactions are described under the heading of the reaction products rather than the type of reaction involved. There are two main exceptions to this: sulphonyl ester displacements are discussed in the Ester Section (6), and a separate Section (22) groups together work on oxidation and reduction. A policy of generous cross-referencing has been adopted. Background references are generally omitted since they are readily located through the primary articles.

The selection of material for the Sections on glycosides and on nucleosides presented particular difficulties, and we have ignored many papers which we judged were concerned mainly with the non-carbohydrate portions of the molecules. That is, we have taken as our guide lines the specific interests of carbohydrate chemists. Reference to papers outside our guide lines can of course be obtained from *Chemical Abstracts*. However, we would draw attention to several detailed papers on plant glycosides from Reichstein's group published in *Helvetica Chimica Acta*.

We have similarly reported nucleoside work that was concerned only with the carbohydrate moeity, and other aspects of these compounds, including cyclonucleosides, are not covered. Thus phosphorylation of nucleosides is reported but the important nucleotides are treated no further. Papers are discussed at length in the appropriate sections [*e.g.* phosphates in Section 6], and Section 21 is essentially a summary of references.

Relatively little work appeared on alditols during 1967 and so reference to them and their derivatives will be found in other Sections as appropriate. However, we have collected all relevant references together in the final Section (27). All aspects of cyclitol chemistry are described in Section 19. Analytical reports are collected in Section 26 and, in general, are not cross-referenced elsewhere. In view of the interest in compounds with elements

other than oxygen in the ring, papers on this topic have been assembled in Section 12, rather than in the Sections on amino- and thio-sugars.

Few papers have reported really novel findings, but an increasing number of authors are taking full advantage of spectroscopic techniques, especially n.m.r., and of separation techniques, particularly preparative t.l.c. and g.l.c. Of particular interest are the first reported uses of 220 Mc./sec. n.m.r. spectra in carbohydrate chemistry, and the resolution of the n.m.r. spectrum of a pyranoid compound into the spectra of the two chair forms by low temperature studies. The development of new routes to D-allose, D-gulose, D-idose, and D-psicose are notable advances, as are new methods for the synthesis of furanosides. Much work on sulphonyl ester displacements still leaves many unexplained features of such reactions. Many sugars of novel structure have been isolated from natural products.

Two textbooks on carbohydrate chemistry have been published in 1967. One was an introductory text for undergraduates,[1] the other a more advanced work.[2] A summary of research on monosaccharide chemistry published during 1966 was reported during 1967,[3] and the annual editions of *Advances in Carbohydrate Chemistry* and *Annual Review of Biochemistry* have appeared.

A paper has been published on the history of the development of the Nomenclature Committee of the Division of Carbohydrate Chemistry of the American Chemical Society,[4] and on its relationship with other similar bodies.

[1] R. J. McIlroy, 'Introduction to Carbohydrate Chemistry,' Butterworths, London, 1967, 133 pp.
[2] E. A. Davidson, 'Carbohydrate Chemistry,' Holt, Rinehart and Winston, New York, 1967, 441 pp.
[3] A. C. Richardson, *Ann. Reports* 1966, **63**, 489.
[4] M. L. Wolfrom, *J. Chem. Doc.*, 1967, **7**, 78.

2
Free Sugars

The chemistry of D-fructose and its derivatives has been reviewed.[5]

Isolation and Synthesis

Several bacteria, identified as genera of *Brevibacterium* and *Corynebacterium*, have been shown to be capable of accumulating large amounts of sugars; these were isolated by paper chromatography and shown to be a mixture of D-*erythro*- and D-*threo*-pent-2-ulose.[6] Xylitol and xylonic acid have been prepared in reasonable yield from D-xylose by the action of *Pichia quercibus* Phaff et Knapp.[7] The synthesis of maltulose by the action of intestinal α-D-glucosidases on sucrose has been reported.[8]

Rare sugars have been made more readily available by new synthetic routes. A convenient synthesis of D-allose has been developed from 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose (see p. 69), and a further report (cf. several in 1966) has appeared on the reduction of 1,2:5,6-di-*O*-isopropylidene-D-*ribo*-hex-3-ulose as a route to the same sugar; the D-*ribo*-hex-3-ulose derivative was also an intermediate in a new synthesis of D-gulose (see p. 129). Two independent reports have described the synthesis of D-psicose starting from 1,2:4,5-diacetals of D-fructose (see p. 178). D-Idose is now readily available from penta-*O*-acetyl-D-glucopyranose (see p. 53). The synthesis of free sugars by degradation of t-butylperoxy-glycosides, aldonic peroxy-esters, and phenylazohydroperoxides, has been described (see pp. 25, 144, and 101, respectively). A detailed study of the electrolytic reduction of D-ribonolactone to D-ribose, an industrial process, has been made (see p. 143).

The compound $(C_6H_{12}O_6)_2$,NaCl,H_2O has been employed for the isolation of α-D-glucopyranose from mutarotated mixtures. Conditions for the extraction of the salt from the complex were described.[9]

A number of papers have appeared on the synthesis of labelled carbohydrates, particularly for use in biosynthetic studies. Derivatives of [6-^{3}H]D-galactose have been prepared in two laboratories, by the oxidation

[5] L. M. J. Verstraeten, *Adv. Carbohydrate Chem.*, 1967, **22**, 230.
[6] M. Misawa, T. Nara, and S. Kinoshita, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 611.
[7] T. Suzuki and H. Onishi, *Agric. and Biol. Chem. (Japan)* 1967, **31**, 1233.
[8] A. Täufel, H. Ruttloff, and K. Täufel, *Carbohydrate Res.*, 1967, **5**, 223.
[9] I. E. Sadovyi, *Zhur. priklad. Khim.*, 1967, **40**, 694.

of D-galactosides with galactose oxidase to the uronic acid derivatives, reduction with sodium borotriti-ide, and hydrolysis by β-galactosidase [10] or by acid.[11] Raffinose with a [6-^{3}H]D-galactose label was prepared in a similar way,[10] as was the derivative of melibi-itol.[11] [1-^{3}H]D-Ribose has been prepared by reduction of 2,3,4,5-tetra-*O*-acetyl-D-ribonyl chloride with lithium aluminium [tri-(t-butoxy)triti-ide], followed by removal of the ester groups.[12] Wilzback tritiations of D-glucose, sucrose, and dextran have been compared, and whereas C-3 was the most readily labelled in the hexose, C-2 appeared the most susceptible in its derivatives.[13] [1-^{14}C]α-L-Fucose has been synthesised from 5-deoxy-L-lyxose (prepared from the calcium salt of L-fuconic acid) by reaction with sodium [^{14}C]cyanide and separation of the epimeric *fuco*- and 6-deoxy-L-*talo*-products. Reduction of the γ-lactone of the former with sodium amalgam gave the required compound in crystalline form.[14]

Aqueous formaldehyde solutions have been shown to be polymerised to monosaccharides by boiling water in the presence of various clays,[15] leading the authors to the conclusion that such a reaction may have provided a route for the prebiotic synthesis of carbohydrates. A similar formaldehyde condensation, in the presence of carbonate-apatite, also yielded monosaccharides,[16] but these authors considered that this was not a good model for prebiotic synthesis since prolonged heating destroyed the products, and because concentrated formaldehyde solutions were required. They suggested [16] that if D-ribose, in particular, was formed by this means, a specific stabilisation mechanism, for example nucleoside formation, must have been available. The formation of carbohydrates from formaldehyde in the presence of tertiary bases such as pyridine and α-picoline has been compared with that using inorganic bases.[17]

Mutarotation

A method for studying the mutarotation of free sugars, based on g.l.c., has been described,[18] and shown to give the same mutarotation coefficients as polarimetric methods. The advantage of the new method is that only very small quantities of sugar are required. Samples of the aqueous solution under study were removed at various times, diluted with DMF, frozen in liquid nitrogen, and then converted into pertrimethylsilyl ether derivatives, which were analysed by g.l.c. The paper[18] described the application to D-glucose, D-galactose, D-arabinose, and D-xylose.

[10] G. Avigad, *Carbohydrate Res.*, 1967, **4**, 430.
[11] J. E. G. Barnett, *Carbohydrate Res.*, 1967, **4**, 267.
[12] R. J. Suhadolnik, T. Uematsu, and R. M. Ramer, *Carbohydrate Res.*, 1967, **5**, 479.
[13] K. H. Ebert and J. Richter, *Z. Naturforsch.*, 1967, *B*, **22**, 788.
[14] H. S. Isbell, H. L. Frush, and N. B. Holt, *J. Res. Nat. Bur. Stand.*, 1967, **71A**, 133.
[15] N. W. Gabel and C. Ponnamperuma, *Nature*, 1967, **216**, 453.
[16] C. Reid and L. E. Orgel, *Nature*, 1967, **216**, 455.
[17] K. Runge and R. Mayer, *Annalen*, 1967, **707**, 161.
[18] R. Bentley and N. Botlock, *Analyt. Biochem.*, 1967, **20**, 312.

Preliminary results on a study of the equilibria between pyranose and furanose forms of a number of aldoses in deuterium oxide by n.m.r. have been reported.[19] No signals corresponding to furanose forms could be detected for D-glucose, D-galactose, D-mannose, D-xylose, D-lyxose, or 2-deoxy-D-*arabino*-hexose; they were shown, however, by solutions of D-allose, D-altrose, D-gulose, D-talose, and D-arabinose. The interaction between the side-chain and the oxygen atom on C-3, when these are *cis*, appeared to be the most unfavourable interaction in the furanose form.

A detailed investigation of the deuterium isotope effects operating during the mutarotation of α-D-xylose, α-D-glucose, and β-D-fructose in deuterium oxide has been made and the results were discussed. The striking parallel between a pyranose ⇌ furanose isomerisation (for fructose) and an α-pyranose ⇌ β-pyranose one (for glucose) indicated that similar rate-determining steps must be present in both.[20] The isotope effect previously observed during the counter-current distribution of [1-^{14}C]D-arabinose was absent with the methyl glycopyranoside and also with [5-^{14}C]D-arabinose. It was therefore proposed[21] that the effect originally observed operated by changing the equilibrium position between the forms of the sugar. The thermodynamics of the mutarotation of α-D-glucopyranose in deuterium oxide have been compared with those for water as solvent; the catalytic functions of D^+, H^+, D_2O, and H_2O were considered.[22]

In a letter which re-stated the evidence that pyranose mutarotation proceeds by way of an acyclic modification, the hypothesis of Christensen, that a C-1 carbanion intermediate is involved, was effectively destroyed.[23]

Physical Measurements

It has been reported that i.r. spectra of carbohydrates were appreciably improved when the measurement was carried out at low temperature.[24] Not only was band definition better, but new bands appeared. The changes were continuous as the temperature was reduced, and were consequently not due to a phase change, but rather to restriction of rotation. The spectra of trehalose dihydrate at 25° and −160° in Nujol were shown.

Stereospecific long-range coupling of the hydroxy-protons in glycopyranoses has been observed. Spectra for DMSO–[$^2H_6$]acetone solutions showed that the effect was present for an axial hydroxy-group, vicinal to an axial proton. Thus, for example, long-range coupling was observed for the C-1-hydroxy-proton in α-D-glucopyranose, but not for the β-anomer.[25] The method should be of great use in studying ketoses, which lack an

[19] S. J. Angyal and V. A. Pickles, *Carbohydrate Res.*, 1967, **4**, 269.
[20] H. S. Isbell and C. W. R. Wade, *J. Res. Nat. Bur. Stand.*, 1967, **71A**, 137.
[21] L. M. Marshall, W. P. Walker, J. A. Gunn, and L. Panton, *J. Chromatog.*, 1967, **29**, 103.
[22] H. Schmid, G. Bauer, and G. Praehauser, *Monatsh.*, 1967, **98**, 165.
[23] F. H. Dean, *J. Colloid Interface Sci.*, 1967, **24**, 281.
[24] J. E. Katon, J. T. Miller, and F. F. Bentley, *Arch. Biochem. Biophys.*, 1967, **121**, 798.
[25] J. C. Jochims, G. Taigel, A. Seeliger, P. Lutz, and H. E. Driesen, *Tetrahedron Letters*, 1967, 4363.

anomeric proton. The broadline n.m.r. spectrum of crystalline α-D-glucose has been studied (at 27 Mc./sec.).[26]

Several papers have appeared on e.s.r. studies on sugar radicals produced in various ways. Those formed when carbohydrates were added to the Ti^{3+}–H_2O_2 system have been studied.[27] Hexitols gave the same spectra, which were different from those given by free sugars. Further, aldoses, ketoses, and glycosides all gave different spectra; attempts were made to analyse the results obtained. The γ-radiation-induced degradation of crystalline mono- and di-saccharides has been investigated,[28] and the products analysed. It was postulated[28] that radicals within irradiated samples react with each other and with dissolved oxygen in aqueous solution. The reactions were found to be strongly dependent on the crystalline form of the sugar. The yields of radicals (from e.s.r.) produced from sugar solutions at low temperatures by irradiation with a ^{60}Co source were higher than expected and a blue colour was formed;[29a] these phenomena were explained by solute-stabilisation of solvated electrons.

The oxidation of monosaccharides by the hydroxy radical has been studied by e.s.r. techniques.[29b]

The polargraphic reduction of a number of monosaccharides has been investigated.[30] One interesting finding was that the reduction characteristics of D-ribose were much more similar to those of its 5-deoxy- than its 4-deoxy-derivative.

Three papers have been published concerning the growth of sucrose crystals from pure and impure solutions,[31] and the intradiffusion coefficients of sucrose in aqueous solution have been measured.[32] Ternary diffusion, refractive index, density, and relative viscosity data have been measured for the system water–sucrose–mannitol, for water–sucrose, and for water–mannitol; various coefficients were calculated and discussed.[33] The phase relations of the systems maltose or lactose–sucrose–water were established at 30°. The solubility of lactose in water was relatively low but increased as the sucrose concentration increased.[34]

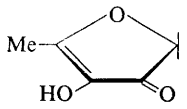
Reactions

Two reports of the epimerisation and rearrangement of D-arabinose in boiling pyridine have appeared.[35, 36] The German workers[36] isolated the

[26] B. J. Poppleton and C. K. Coogan, *Carbohydrate Res.*, 1967, **3**, 505.
[27] P. J. Baugh, O. Hinojosa, and J. C. Arthur, *J. Phys. Chem.*, 1967, **71**, 1135.
[28] G. Löfroth, *Acta Chem. Scand.*, 1967, **21**, 1997.
[29a] W. R. Elliott, *Science*, 1967, **157**, 558.
[29b] R. O. C. Norman and R. J. Pritchett, *J. Chem. Soc.* (*B*), 1967, 1329.
[30] B. Capon, A. A. Levy, and W. G. Overend, *Carbohydrate Res.*, 1967, **5**, 93.
[31] B. M. Smythe, *Austral. J. Chem.*, 1967, **20**, 1087, 1097, 1115.
[32] J. F. Tilley and R. Mills, *J. Phys. Chem.*, 1967, **71**, 2756.
[33] H. D. Ellerton and P. J. Dunlop, *J. Phys. Chem.*, 1967, **71**, 1291.
[34] F. H. C. Kelly, *J. Appl. Chem.*, 1967, **17**, 223.
[35] M. Fedoroňko and K. Linek, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2177.
[36] H. Zinner and W. Rehpenning, *Carbohydrate Res.*, 1967, **5**, 176.

starting sugar, D-ribose and D-*erythro*-pent-2-ulose, by ion-exchange chromatography (bisulphite form). The ketose was converted into several arylsulphonylhydrazones, and into 1,3,4,5-tetra-*O*-acetyl-*keto*-D-*erythro*-pentulose, further characterised as its dimethyl dithioacetal. The Czech workers using identical reaction conditions claimed [35] that the reaction mixture contained all four D-aldopentoses, D-*erythro*- and D-*threo*-pent-2-ulose and D-*erythro*- and D-*threo*-pentul-3-ose. Similar results were also obtained from the same treatment of D-xylose.[35] The isolation of the above products was considered to be verification of an ene-diol mechanism. A detailed kinetic study, including the determination of thermodynamic parameters, has been made of the D-mannose to D-fructose isomerisation that is catalysed by a mannose isomerase from *Streptomyces aerocolorigenes*.[37] Epimerisation of lactose by calcium hydroxide gives lactulose, which it is now reported[38a] can easily be obtained in pure form by crystallisation from a syrup containing only 50% of the ketose, using methanol. An enzyme from *Ruminococcus albus* has been shown to catalyse the interconversion of cellobiose and 4-*O*-(β-D-glucopyranosyl)-D-mannose.[38b]

D-Glucose, when kept at 190°, decomposed with the formation of 5-(hydroxymethyl)-2-furfuraldehyde. The decomposition was catalysed by added glass, the effect being directly proportional to the cation content and independent of the surface area of the glass.[39] A preliminary report has appeared on the study of the thermal decomposition of D-glucose using ^{14}C-labelled material. The main carbon atoms contributing to the volatile products (carbon dioxide and monoxide) were shown to be C-1 and C-2, the former being dominant.[40] The decomposition (chemically unspecified) of D-glucose has been investigated over the pH range 0–10 and at varying temperatures (80–120°); the decomposition was minimal at pH 3·5. D-Fructose and invert sugar were also studied.[41] Factors influencing the production of chromophores on thermal and acid-catalysed degradation of D-ribose and its 2-deoxy-derivative have been examined, and have enabled spectrophotometric methods for analyses of both sugars to be developed.[42a,42b] Pentoses when heated with amine acetates gave a compound believed [43] to have structure (1).



(1)

[37] Y. Takasaki, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 435.
[38a] B. J. Oosten, *Rec. Trav. chim.*, 1967, **86**, 673, 675.
[38b] T. R. Tyler and J. M. Leatherwood, *Arch. Biochem. Biophys.*, 1967, **119**, 363.
[39] F. Orsi, *Magyar Kem. Folyóirat*, 1967, **73**, 1.
[40] Y. Houminer and S. Patai, *Tetrahedron Letters*, 1967, 1297.
[41] A. R. Sapronov and S. E. Kharin, *Sakharn. Prom.*, 1967, **41**, 55.
[42a] E. R. Garrett, J. Blanch, and J. K. Seydel, *J. Pharm. Sci.*, 1967, **56**, 1560.
[42b] J. K. Seydel, E. R. Garrett, W. Diller, and K.-J. Schaper, *J. Pharm. Sci.*, 1967, **56**, 858.
[43] T. Severin and W. Seilmeier, *Z. Lebensm.-Untersuch.*, 1967, **134**, 230.

Two papers have described the degradation of D-fructose.[44,45] Acid-catalysed decomposition has been studied[44] at pH 1·0, 2·15, and 3·5, and it has been shown that the major products formed were 5-(hydroxymethyl)-2-furfuraldehyde and 2-(hydroxyacetyl)furan together with several minor products, which varied with varying pH. It was suggested that the products were formed by a series of enolisation and dehydration steps. Prolonged refluxing of D-fructose with 10% sulphuric acid has been shown[45] to give levulinic acid and acetone (isolated as their 2,4-dinitrophenylhydrazones) and carbon dioxide as the only identifiable products.

The self-condensation reactions of D-glucose, D-mannose, and D-galactose and of some of their derivatives have been examined[46a] in the absence of a catalyst. Above 220°, intramolecular condensation predominated, below that temperature, intermolecular condensation was dominant. The degree of polymerisation for the sugars was found to be D-galactose > D-mannose > D-glucose. It was also found that the hydroxy-group on C-1 must be free for condensation to occur.

Brewer's yeast, which showed α-, but not β-galactosidase activity, has been shown to catalyse the polymerisation of D-galactose.[46b] The predominant oligosaccharide product was 6-*O*-(α-D-galactopyranosyl)-D-galactose with smaller proportions of α(1 → 3), α(1 → 4), and α(1 → 5) linked D-galactobioses.

The reversion of D-glucose as a function of temperature (40–160°), pH(1–6), glucose concentration (10–80%), and time (0·5–100 hr.) has been studied.[47] Reaction was found to be appreciable for solutions greater than 40% in sugar and then di- and tri-saccharides were obtained; it was assumed that 1,6-linkages were formed. The reversion was accompanied by condensation between D-glucose and 5-(hydroxymethyl)-2-furfuraldehyde.

From a study[48] of the kinetics of the hydrogenation of D-glucose over a variety of catalysts, it was concluded that catalysts composed of 0·1–0·5% ruthenium in nickel, or 5% palladium in nickel were the most effective. However, since it was stated that increasing the pH of the medium to 12 facilitated reduction, it must be assumed that reactions other than simple reduction were involved.

The oxidation of D-glucose, D-galactose, D-xylose, L-arabinose, and lactose with bromine at different pH values has been studied. Optimum rates occurred at pH 7·9, and hypobromous acid was identified as the oxidant.[49] Oxidation of D-glucose to D-gluconic acid in the presence of

[44] P. E. Shaw, J. H. Tatum, and R. E. Berry, *Carbohydrate Res.*, 1967, **5**, 266.
[45] E. S. Amin, *Carbohydrate Res.*, 1967, **4**, 96.
[46a] J. W. Liskowitz and B. Carroll, *Carbohydrate Res.*, 1967, **5**, 245.
[46b] M. J. Clancy and W. J. Whelan, *Arch. Biochem. Biophys.*, 1967, **118**, 724.
[47] A. Sroczynski and M. Boruch, *Zeszyty Nauk Politech. lodz.* (*Chem.*), 1967, **12**, 61.
[48] F. B. Bizhanov, D. V. Sokol'skii, N. I. Popov, N. Ya. Malkina, and A. M. Khisametdinov, *Kinetika i Kataliz*, 1967, **8**, 620.
[49] N. Bhattacharya and M. L. Sen Gupta, *Indian J. Chem.*, 1967, **5**, 554.

palladium on barium sulphate has been explored as a possible commercial process.[50]

The structures of the polymeric products obtained on condensing D-glucose with fluorene have been investigated, together with similar products from diphenylmethane, diphenylethane, triphenylmethane, and biphenyl.[51]

Several papers have appeared on the Maillard reaction. The nature of the carbonyl compounds produced was dependent on the amino-acid used (for example, L-alanine gave acetaldehyde, L-phenylalanine gave phenylacetaldehyde, and L-valine gave isobutyraldehyde), whereas the rate and extent of production of the carbonyl compound depended on the sugar used; D-xylose was the most reactive, then D-glucose and maltose.[52] It has been shown[53] that the rate of production of carbon dioxide depended largely on the aldose involved. The gas was liberated faster with pentoses than with hexoses or disaccharides. The main degradation route was by the Strecker pathway, but an additional, unidentified pathway appeared to exist. Bisulphite and thiols have been found to inhibit the early stages of the Maillard reaction, whereas benzoyl peroxide and disulphides affect the browning step. Theories of the mechanism of inhibition by sulphites are discussed.[54]

A quantitative study has been made of the reaction of D-fructose with amino-acids. Products with the *manno*-configuration arose before *gluco* ones, but the former fall off so that subsequently the latter predominated.[55]

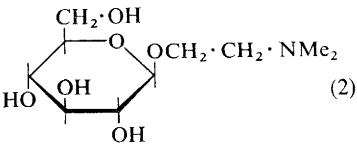
[50] J. Okada, S. Morita, Y. Matsuda, and T. Takenawa, *J. Pharm. Soc. Japan*, 1967, **87**, 1326.
[51] F. Micheel and H. Licht, *Makromol. Chem.*, 1967, **103**, 91.
[52] L. W. Rooney, A. Salem, and J. A. Johnson, *Cereal Chem.*, 1967, **44**, 539.
[53] S. J. Cole, *J. Food Sci.*, 1967, **32**, 245.
[54] P.-S. Song and C. O. Chichester, *J. Food Sci.*, 1967, **32**, 98, 107.
[55] K. Heyns, G. Müller, and H. Paulsen, *Annalen*, 1967, **703**, 202.

3
Glycosides

O-Glycosides

Synthesis.—Reaction of free sugars in aqueous solution with methanolic diazomethane provides a method for the synthesis of methyl glycosides. Methyl β-D-glucofuranoside and -pyranoside were obtained from D-glucose, the corresponding glycosides were produced from D-galactose, and the α-pyranoside was obtained from D-mannose.[56] Complete methylation of free sugars has been shown to occur with methyl iodide in DMF in the presence of barium oxide and hydroxide. The mixture of isomers obtained from D-galactose has been analysed by the combined use of g.l.c. and mass spectrometry, and found to consist mainly of the methylated furanosides and to be devoid of septanosides.[57] The mass spectra of the α-anomers of the five-, six-, and seven-membered glycosides were recorded and were seen to differ appreciably.[57] This further illustrates how mass spectrometry is applicable to the determination of ring size of glycosides.

Standard routes to vinyl ethers afford means for the vinylation of hydroxy-groups at positions other than the anomeric centre in free sugars, but the sensitivity of these compounds to the required conditions usually precludes the possibility of synthesising vinyl glycosides directly. Indirect



(2)

routes to vinyl glucopyranosides have now been reported.[58] 2-Dimethylaminoethyl β-D-glucopyranoside (2) was prepared by way of the 2-chloroethyl glycoside, and its acetate was found to be unsuitable for application of the Cope degradation. However, the derived quaternary ammonium hydroxide underwent the Hofmann degradation to give vinyl β-D-glucopyranoside which was isolated as its tetra-acetate. An alternative route to

[56] E. Klajn and A. Damanski, *Bull. Soc. Chim. biol.*, 1967, **49**, 521.
[57] K. Heyns, D. Müller, R. Stute, and H. Paulsen, *Chem. Ber.*, 1967, **100**, 2664.
[58] T. D. Perrine, C. P. J. Glaudemans, R. K. Ness, J. Kyle, and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 664.

the anomeric vinyl glucosides was established by using the mercuric acetate-catalysed transvinylation reaction. To reduce the likelihood of oxidation, tetra-*O*-benzyl-α-D-glucopyranose was employed, and this, on treatment with excess of boiling isobutyl vinyl ether, in the presence of the catalyst, afforded a mixture of glycosides which were separated by column chromatography and debenzylated with sodium in liquid ammonia. The pure products were obtained (α crystalline, β syrup) by successive acetylation and deacetylation. These vinyl glycosides resembled the corresponding phenyl compounds in that the α-anomer was relatively stable in alkali (the β-anomer gave the 1,6-anhydride) and, furthermore, the tetrabenzyl α-glycoside was the more labile in acid.[58]

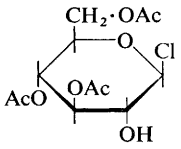
The preparation of several aryl glycosides by established methods has been reported. As part of a study of the substrate specificity of α-L-rhamnosidase, the synthesis of a variety of aryl α-L-rhamnopyranosides was undertaken.[59] The Helferich fusion method using L-rhamnose tetra-acetate and catalysts such as toluene-*p*-sulphonic acid or zinc chloride was found to be superior to the Koenigs–Knorr approach using mercury salts. Similarly,[60] 2,3,4,6-tetra-*O*-acetyl derivatives of phenyl α-D-galacto-pyranosides were obtained in 40–50% yield on heating D-galactose penta-acetate with phenols in the presence of zinc chloride in acetic acid and acetic anhydride. Deacetylation proceeded smoothly with sodium methoxide and the following α-D-galactopyranosides were obtained: phenyl, *o*-, *m*-, and *p*-cresyl, *o*- and *p*-nitrophenyl, and *p*-acetylphenyl. Reaction of penta-*O*-acetyl-β-D-glucopyranose with phenol in benzene solution in the presence of stannic chloride has been previously shown to afford the aryl β-glycoside derivative. It has now been reported [61] that the α- and β-anomers were found in the ratio 3 : 7, and that the α-anomer may be isolated using fractional crystallisation and column chromatography. The acetates of phenyl α-D-galactopyranoside, *o*-nitrophenyl 1-thio-α-D-gluco- and D-galactopyranoside were also isolated by these methods.[61] The preparation of a number of phenyl β-D-xylopyranosides has also been reported.[62] In related work the range of aromatic glycosides has been extended to include azulene and ferrocene derivatives as well as phenyl and thiophenyl compounds.[63] Practical details have been described for optimising the yield of benzyl β-L-arabinopyranoside obtained by the ordinary Fischer method.[64]

The synthesis of the anomeric phenyl glycosides of D-neuraminic acid was achieved using the peracetylated glycosyl chloride and phenol in the presence of silver carbonate, followed by deacetylation. The more dextrorotatory isomer was assigned the α-configuration and was cleaved by

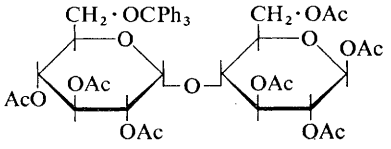
[59] S. Kamiya, S. Esaki, and M. Hama, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 397.
[60] P. M. Dey, *Chem. and Ind.*, 1967, 1637.
[61] J. L. Bose and T. R. Ingle, *Chem. and Ind.*, 1967, 1451.
[62] C. K. De Bruyne and F. Van Wijnendaele, *Carbohydrate Res.*, 1967, **4**, 102.
[63] W. Treibs, *Chimia* (*Switz.*), 1967, **21**, 537.
[64] J. E. McCormick, *Carbohydrate Res.*, 1967, **4**, 263.

neuraminidase. Crystalline methyl esters and acetyl derivatives were described.[65a] Methyl glycosides have also been described.[65b] Aryl glycosides can also be synthesised enzymically using a preparation obtained from *Aspergillus niger*. With it, α-D-glucopyranosyl units from maltose were transferred to phenols and also to alcohols and carboxylic acids.[66]

The Koenigs–Knorr reaction continues to be applied in the synthesis of a variety of glycosyl derivatives and is also referred to elsewhere in this report (see p. 00). The usefulness of β-acylglucosyl halides in α-glucoside synthesis is curtailed when the acyl function at C-2 participates in the reaction. In the absence of such groups inversion occurs smoothly.



(3)



(4)

3,4,6-Tri-*O*-acetyl-β-D-glucopyranosyl chloride (3) has been used[67] to prepare 3,4,6-tri-*O*-acetyl-1-*O*-(2,4,6-trimethylbenzoyl)-α-D-glucose in 76% yield, and the corresponding 1-nitrate in 45% yield. The latter compound was also synthesised by the use of 3,4,6-tri-*O*-acetyl-2-*O*-trichloroacetyl-β-D-glucopyranosyl chloride in the presence of an alcohol to solvolyse the trichloroacetate. Treatment of compound (3) with alcohols in the presence of zinc chloride gave β-glycosides, presumably as a result of prior anomerisation of the halide.[67] A chemical synthesis of panose [*O*-α-D-glucopyranosyl(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose] was undertaken[68a] to confirm the structure of the trisaccharide. Condensation of 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride, an α-glucosylating agent, with the trityl-maltose hepta-acetate (4) in nitromethane in the presence of silver perchlorate and a drying agent afforded, after hydrogenolysis and deacetylation, panose (17%) together with the 1 → 6-β-linked isomer. Compound (4) was prepared by treatment of maltose with a limited amount of trityl chloride, acetylation of the products and fractionation of the mixture on silica gel. Lower yields of panose were obtained when detritylated compounds were used, so that the leaving-group properties of the trityl carbonium ion are apparently advantageous.[68a] It was tentatively suggested[68a] that an essentially unimolecular process was involved, and the cyclic carbonium-oxonium ion was depicted in the 1H-half-chair conformation, although no evidence was produced to substantiate this. The departing halide was believed to exert some steric control. The synthesis of

[65a] P. Meindl and H. Tuppy, *Monatsh.*, 1967, **98**, 53.
[65b] W. Gielen, *Hoppe-Seyler's Z. Physiol. Chem.*, 1967, **348**, 378.
[66] S. M. Hopkinson and J. B. Pridham, *Biochem. J.*, 1967, **105**, 655.
[67] B. Helferich and D. Arndt, *Chem. Ber.*, 1967, **100**, 2117.
[68a] M. L. Wolfrom and K. Koizumi, *J. Org. Chem.*, 1967, **32**, 656.

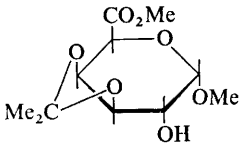
6-*O*-(α-D-xylopyranosyl)-D-mannose was achieved by use of the non-participating chlorosulphate group.[68b]

Straightforward Koenigs–Knorr condensations have been used in the synthesis of 3-*O*-(β-D-glucopyranosyl)-D-galactose,[69,70] of its β-methyl glycoside,[69] and of the corresponding glucopyranosyluronic acid,[70] and of 2-*O*-(α-L-fucopyranosyl)-L-fucopyranose,[71] 2-*O*-(α-L-fucopyranosyl)-D-galactose,[72] and 3,6-anhydro-4-*O*-(β-D-galactopyranosyl)-D-galactose.[73a]

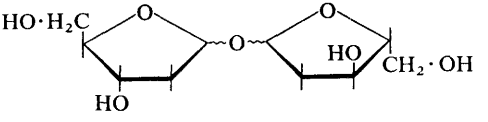
The use of the greater reactivity of the C-3 hydroxy-group as compared to the C-4 hydroxy-group in the galactose moiety [70] has led to the synthesis of the trisaccharide, *O*-β-D-galactopyranosyl(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose by selective glycosidation of benzyl 4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside with tetra-*O*-acetyl-α-D-galactopyranosyl bromide.[73b]

By use of the Bredereck or Helferich procedures the following L-rhamnose disaccharides have been synthesised: 6-*O*-(α-L-rhamnopyranosyl)-D-glucose, 6-*O*-(α-L-rhamnopyranosyl)-D-galactose, 2-*O*-(α-L-rhamnopyranosyl)-D-glucose, 6-*O*-(α-L-rhamnopyranosyl)-D-mannose, and 1-*O*-(α-L-rhamnopyranosyl)-D-fructose.[74]

The synthesis of 2,3,4-tri-*O*-acetyl-6-*O*-(benzylthio)carbonyl-α-D-galactopyranosyl bromide has been accomplished from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, and from it, β-glycosides were obtained. This work opened the way to the synthesis of complex 6-substituted galactosides and several new compounds were described.[75] The direct synthesis of a pseudoaldobiouronic acid with an α-1 → 2-linkage has been reported; reaction of the methyl ester methyl galacturonide (**5**) with



(5)



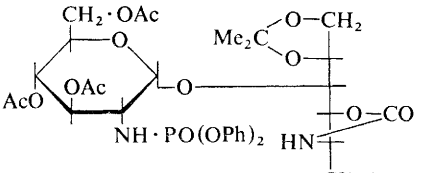
(6)

tetra-*O*-acetyl-α-D-glucopyranosyl bromide in chloroform, in the presence of mercuric cyanide, gave the required disaccharide [76] in low yield. The unusual 2-deoxy-D-*erythro*-pentofuranosyl 2-deoxy-D-*erythro*-pentofuranoside (6), obtained in the course of the synthesis of aza-analogues of

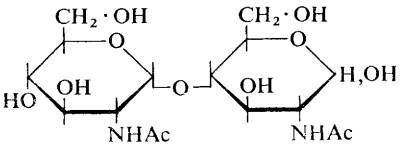
[68b] H. J. Jennings, *Chem. Comm.*, 1967, 722.
[69] A. Stoffyn and P. Stoffyn, *J. Org. Chem.*, 1967, **32**, 4001.
[70] H. M. Flowers, *Carbohydrate Res.*, 1967, **4**, 312.
[71] H. M. Flowers, A. Levy, and N. Sharon, *Carbohydrate Res.*, 1967, **4**, 189.
[72] A. Levy, H. M. Flowers, and N. Sharon, *Carbohydrate Res.*, 1967, **4**, 305.
[73a] S. Hirase and C. Araki, *Bull. Chem. Soc. Japan*, 1967, **40**, 2627.
[73b] D. Beith-Halahmi, H. M. Flowers, and D. Shapiro, *Carbohydrate Res.*, 1967, **5**, 25.
[74] S. Kamiya, S. Esaki, and M. Hama, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 261.
[75] H. Libert and L. Schmid, *Monatsh.*, 1967, **98**, 1375.
[76] P. Šipoš and Š. Bauer, *Tetrahedron Letters*, 1967, 443.

2-deoxynucleosides, has been prepared by condensation of 2-deoxy-3,5-di-*O*-toluyl-D-*erythro*-pentosyl chloride with the corresponding free sugar.[77] In other applications of the Koenigs–Knorr synthesis, a number of substituted alkyl β-D-xylopyranosides,[78] dihalogenopropyl and dihydroxypropyl D-glucosides,[79] glucosides of hydroxy- and mercapto-cinnoline,[80] and tetradecyl glycosides of β-maltose and of 3-*O*-(α-D-galactopyranosyl) D-galactose [81] have been prepared. In a more complex example, acetylated D-glucopyranosyl and D-galactopyranosyl bromides have been condensed with 3-*O*-benzoyl-*N*-octadecanoyl-1-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-DL-dihydrosphingosine to give dihydroceromide dihexosides with β-1,4-linkages.[82]

Amino-sugar disaccharides have been synthesised by the Koenigs–Knorr method; treatment of 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal with 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoroylamino-α-D-glucopyranosyl bromide in the presence of mercuric cyanide gave the α- (7) and β-disaccharide products (35 and 10%). From the former, by a sequence of deblocking reactions, crystalline 2-acetamido-4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-glucose (8) was obtained.[83] A different glucosylating agent, 3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-α-D-glucopyranosyl bromide has



(7)



(8)

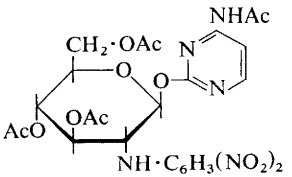
been used in the preparation of 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose, a unit found in biologically important polymers.[84] Reaction of the same glucosylating agent with 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose gave the required (1 → 4)-linked disaccharide, as well as appreciable amounts of the (1 → 3)-linked isomer, showing there was no strong preference for substitution at the equatorial position [85] (cf. ref. 70, 73*b*, and p. 27).

αα-Trehalosamine, which showed antibacterial activity, has been synthesised by condensation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose

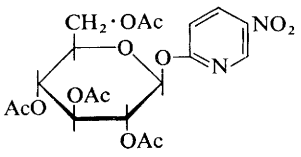
[77] J. Pliml and F. Šorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3060.
[78] C. K. De Bruyne and G. Van der Groen, *Carbohydrate Res.*, 1967, **5**, 95.
[79] F. Marquez and A. Gonzalez, *Anales real Soc. espaň. Fis. Quim.*, 1967, **63B**, 1137.
[80] G. Wagner and D. Heller, *Arch. Pharm.*, 1967, **300**, 783.
[81] J. Koscielak and R. W. Jeanloz, *Carbohydrate Res.*, 1967, **5**, 220.
[82] H. M. Flowers, *Carbohydrate Res.*, 1967, **4**, 42.
[83] K. Heyns, K. Propp, R. Harrison, and H. Paulsen, *Chem. Ber.*, 1967, **100**, 2655.
[84] A. J. Acher and D. Shapiro, *Israel J. Chem.*, 1967, **5**, 61P.
[85] D. Shapiro, A. J. Acher, and E. S. Rachaman, *J. Org. Chem.*, 1967, **32**, 3767.

and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-α-D-glucopyranosyl bromide.[86] The structure α-D-mannopyranosyl 2-amino-2-deoxy-α-D-glucopyranoside has been assigned to a naturally occurring antibiotic (see p. 162).

In some instances, attempted nucleoside syntheses involving the use of glycosyl halide derivatives gave *O*-glycosides. For example, the silver salt of *N*-acetylcytosine, when condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,4′-dinitrophenylamino)-α-D-glucopyranosyl chloride, gave predominantly the β-*O*-glycoside (9) which was also obtained by acetylation of the analogous compound prepared using the silver salt of cytosine. This same compound (9) was also formed when the mercury salt of *N*-acetylcytosine was employed, although previously this salt yielded *N*-glycosides exclusively. *O*- To *N*-rearrangement did not occur on heating the *O*-glycoside



(9)



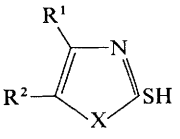
(10)

in xylene in the presence of mercuric bromide.[87] Similar condensation between tetra-*O*-acetyl-α-D-glucopyranosyl bromide and the silver salt of 5-nitro-2-pyridone gave the β-*O*-glycoside (10); this isomerised on treatment with mercuric bromide in toluene to the α-*O*-glycoside and the β-*N*-isomer. Similar rearrangements could also be induced with Lewis acids. The absence of α-*N*-glycosides was attributed to participation of the C-2-acetoxy-group in the reaction.[88]

In a closely related study, the isomerisations of *O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-2-hydroxypyridines substituted at C-5 have been investigated. Anomerisations and *O*- to *N*-migrations compete and relative rates were dependent upon the nature of the 5-substituent.[89] Glucosylation and thioglucosylation of the heterocyclic compounds (11) were examined under different conditions, and the position of substitution and the anomeric configuration of the products were discussed. Increase in the size of R favoured *S*-glycosylation.[90] This type of work has been extended by the synthesis of ribopyranosides and ribofuranosides of 2-hydroxypyridine and the study of the *O*- to *N*-migrations of the products.[91]

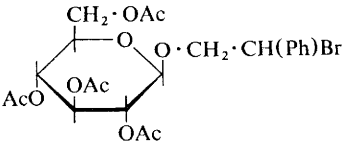
[86] S. Umezawa, K. Tatsuta, and R. Muto, *J. Antibiotics* (*Tokyo*) *Ser. A*, 1967, **20**, 388.
[87] H. G. Garg and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1967, 51.
[88] D. Thacker and T. L. V. Ulbricht, *Chem. Comm.*, 1967, 122.
[89] G. Wagner and H. Gentzsch, *Z. Chem.*, 1967, **7**, 310.
[90] P. Nuhn and G. Wagner, *Z. Chem.*, 1967, **7**, 187.
[91] H. Pischel and G. Wagner, *Arch. Pharm.*, 1967, **300**, 602.

Styrene oxide is a further compound which can undergo glycosylation at either of two centres and the crystalline product obtained after reaction with tetra-*O*-acetyl-D-glucopyranosyl bromide was believed to be the 2-bromo-2-phenylethyl isomer (12).[92] (This formula was drawn in the paper as the β-L-form, and the stereochemistry at the new asymmetric centre was not defined.)



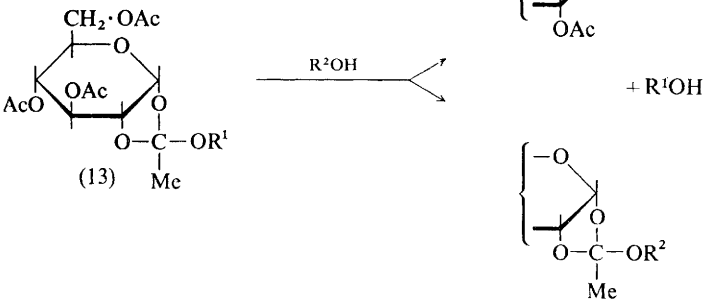
(11)

X = NMe, O, or S
R^1, R^2 = H, Me, or Ph



(12)

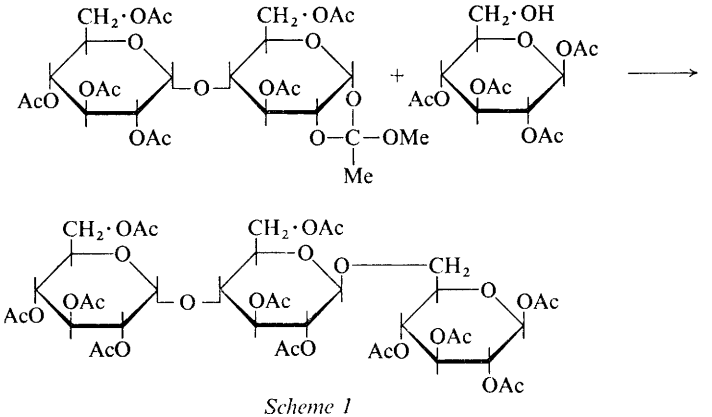
The synthesis of glycosides from orthoesters, which promises to rival the Koenigs–Knorr reaction, has been studied in detail, and a full report of its application to di- and tri-saccharide synthesis has appeared.[93] Reaction of an alcohol with a glucosyl 1,2-orthoester, for example (13) can lead to two products, as shown, *via* an intermediate cyclic acetoxonium ion, one being the 1,2-*trans*-glycoside (14). The Russian workers[93]



examined the effects of solvents and various catalysts on the reaction and found that a standard method for glycosylation was attained by using boiling nitromethane as solvent and mercuric bromide as catalyst. They illustrated the method by the synthesis of several disaccharides (some furanosyl compounds) and a trisaccharide. Variable, but in some cases excellent, yields were obtained. It was suggested that $HgBr_3^-$ forms an ion pair with the acyloxonium ion in polar solvents and thus shields it

[92] F. Marquez and A. Gonzalez, *Anales real Soc. espaň. Fis. Quim.*, 1967, **63(B)**, 491.
[93] N. K. Kochetkov, A. J. Khorlin, and A. F. Bochkov, *Tetrahedron*, 1967, **23**, 693.

from direct attack which would regenerate an orthoester. Orthobenzoates were found to be better for this purpose than orthoacetates and yields of products varied according to the nature of the nucleophilic hydroxy-group, primary being more suitable than secondary ones. The synthesis of 1,2-orthoesters previously developed by this group of workers was discussed with respect to their use in this reaction.[93] Portions of this work including the trisaccharide synthesis (Scheme 1) have been described in



Scheme 1

Russian,[94, 95] and the method has been applied by other workers to the synthesis of the 1-*O*-(β-D-galactofuranosyl)-D-glyceritol, shown to be identical to the natural material obtained from bacterial lipid fractions.[96] Acidic treatment of glycosyl 1,2-orthoesters also affords glycosides (see p. 49) and Kochetkov and his group have developed this into a suitable modification of their original procedure and have described the preparation by this means of β-gentiobiose octa-acetate.[97]

Hydrolysis and Anomerisation.—The acid-catalysed hydrolysis of glycosides has been reviewed.[98] Several reports have appeared of further studies on the hydrolysis of a variety of glycosides and a review on the subject was presented at a discussion on the structure and function of lysozyme.[99]

The mechanism of the acidic hydrolysis of a series of aldofuranosides has been studied and discussed in relationship to the reaction of pyranosides.[100] Glycosyl–oxygen fission occurred following a fast initial proton

[94] N. K. Kochetkov, A. Ya. Khorlin, and A. F. Bochkov, *Zhur. obshchei Khim.*, 1967, **37**, 338.
[95] N. K. Kochetkov, A. Ya. Khorlin, A. F. Bochkov, L. B. Demushkina, and I. O. Zolotuchin, *Zhur. obshchei Khim.*, 1967, **37**, 1272.
[96] H. F. G. Beving, H. B. Boren, and P. J. Garegg, *Acta Chem. Scand.*, 1967, **21**, 2083.
[97] A. F. Bochkov, V. I. Snyatkova, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2684.
[98] J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1967, **22**, 25.
[99] C. A. Vernon, *Proc. Roy. Soc.*, 1967, *B*, **167**, 389.
[100] B. Capon and D Thacker, *J. Chem. Soc.* (*B*), 1967, 185.

transfer, and the negative entropies of activation were interpreted as indicating either a bimolecular displacement by water of the protonated aglycone, or a ring-opening process. Participation in the hydrolyses by hydroxy-groups within the molecules was discussed and considered to be unimportant. The reactions are therefore fundamentally different from those of pyranosides, studies on which are usually carried out by varying the aglycones or the configurations at the asymmetric centres of the sugar and these have produced evidence which favours the *A*-1 mechanism in which the rate-determining step is the unimolecular cleavage of the glycosyl–oxygen bond. The investigation of the hydrolysis of methyl 2-chloro-2-deoxy-β-D-glucopyranoside in which the electron-withdrawing group at C-2 should favour a bimolecular mechanism has now been reported.[101] Kinetic evidence showed that chlorine relative to hydrogen reduced the rate of the hydrolysis but again indicated a unimolecular process. However, the entropy of activation was appreciably less than that reported for the unsubstituted glucoside and was interpreted as indicating the incursion of some *A*-2 character into the mechanism. A survey has been made of the kinetic data available on the hydrolysis of methyl α- and β-D-glucopyranoside.[102]

A kinetic investigation has also been undertaken of the hydrolysis of 2-, 3-, 4-, and 6-*O*-methyl ethers of methyl β-D-glucopyranoside and of gentiobiose. In both series rates were reduced by substitution, the sequence of effects being $3>2>4>6$, and it was concluded that the results were consistent with the cyclic carbonium-ion mechanism for the hydrolysis.[103a]

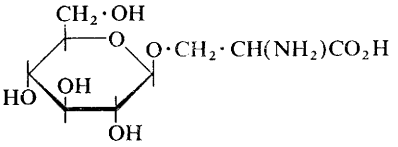
Studies of the kinetics and products of hydrolysis and acetolysis of [1-^{14}C]cellotriose have suggested that the mechanisms of acid hydrolysis and acetolysis may be different.[103b] In acid hydrolysis with 50% sulphuric acid at 30° or with 0·5N-sulphuric acid at both 90° and 120° the ratio of the rate of hydrolysis of the glycosidic bond at the non-reducing end of the trisaccharide to that of the reducing end was 1·5. In acetolysis the ratio was reversed and a threefold preference was observed for cleavage of the glycosidic bond at the reducing end of the trisaccharide.

A study of the hydrolysis of *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-D-glucopyranosides and *o*- and *p*-nitrophenyl D-glucosides has been reported[104] between pH 0·75 and 11·72. Spontaneous hydrolysis occurred only for the β-anomers; the α-compounds showed specific acid and specific base catalysis. Participation of the acetamido-group and of the C-2-hydroxy-group accounted for the spontaneous hydrolysis of the β-isomers and the amide function was found to be 10^3 times more effective than the hydroxy-group. Details of all the reactions were discussed in full, mechanisms were proposed, and the mode of action of the glycosidase lysozyme

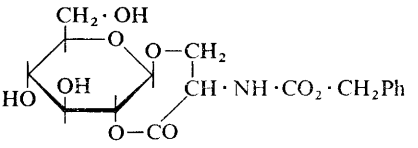
[101] E. Buncel and P. R. Bradley, *Canad. J. Chem.*, 1967, **45**, 515.
[102] J. Szejtli, *Stärke*, 1967, **19**, 173.
[103a] K. K. De and T. E. Timell, *Carbohydrate Res.*, 1967, **4**, 72.
[103b] M. S. Feather and J. F. Harris, *J. Amer. Chem. Soc.*, 1967, **89**, 5661.
[104] D. Piszkiewicz and T. C. Bruice, *J. Amer. Chem. Soc.*, 1967, **89**, 6237.

was considered in the light of the findings.[104] The hydrolysis under acid and basic conditions of glucopyranosyloxy-π-deficient heteroaromatic compounds has been studied.[105]

Because of their biochemical significance the glycosides of L-serine have received attention. Reactions of the serine moiety of *O*-β-D-glucopyranosyl-L-serine (15) were reported, and the intramolecular lactone (16) has been

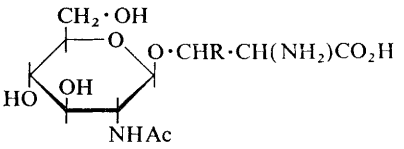


(15)



(16)

subjected to periodate oxidation. Cleavage and subsequent reduction and hydrolysis occurred as expected.[106] In acidic media the hydrolysis of (15) was comparable with alkyl glycoside cleavage, but the glycoside bond was somewhat stabilised by the amino-group.[107] In alkaline solution, however, glycoside hydrolysis of serine esters occurred by a β-elimination mechanism, so such derivatives were appreciably more sensitive than alkyl analogues. The rates of glucosylserine ester hydrolysis and glycoside cleavage were compared, and it was noted that the free acid (carboxylate ion) is stable under these conditions.[107] In similar work it has been shown that *O*-seryl and *O*-threonyl 2-acetamido-2-deoxy-β-D-glucosides (17) were

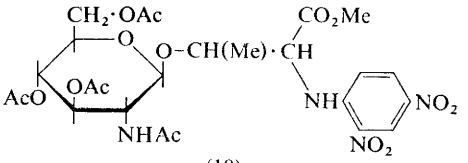


(17) R = H or Me

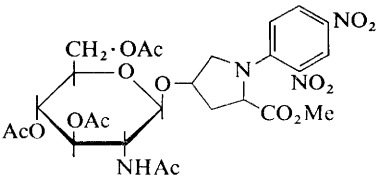
stable in alkaline solution, but that substitution in the carboxylate group rendered them labile, as would be expected.[108] Absence of alkaline degradation of glycopeptides containing hydroxyamino-acids therefore cannot be

[105] G. Wagner and H. Frenzel, *Arch. Pharm.*, 1967, **300**, 591; *Pharmazie*, 1967, **22**, 415.
[106] V. A. Derevitskaya and M. G. Vafina, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 1528.
[107] N. K. Kochetkov, M. G. Vafina, and V. A. Derevitskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 1535.
[108] J. Montreuil, M. Monsigny, and M.-T. Buchet, *Compt. rend.*, 1967, **D, 264**, 2068.

taken as proof of the absence of glycoside bonds. A concurrent report has been given of the alkaline lability of the related threonine glycoside (18),[109] but the proline analogue (19) cannot undergo a β-elimination and was base stable.[110]



(18)



(19)

Considerable attention has been given to the hydrolysis of uronosides. A further study of the acid hydrolysis of β-D-glucuronosides (methyl, ethyl, isopropyl, butyl, s-butyl, and phenyl) was conducted at different acid concentrations, and the conclusion was reached that an *A*-1 mechanism, that is that followed by the corresponding glycopyranosides, applied.[111] Electron-attracting groups in the aglycone decreased the rate of hydrolysis, the plot of log k/k_0 against σ^* being linear. With the glucosides, however, the minimum rate was found for the methyl aglycone and both electron-releasing and electron-withdrawing groups facilitated the hydrolysis.[112] Other workers in very similar studies have investigated the hydrolysis of a number of β-D-glucopyranosiduronic acids with aglycones of different electron affinities (mainly alkyl and 2-substituted ethyl).[113] It was found that the energies and entropies of activation were lower than those for the corresponding β-D-glucopyranosides. For the acids the rates were inversely proportional to the electron affinities of the aglycones, whereas the rates of hydrolysis of the glucopyranosides were reported to be independent of the polarity of the aglycone.

In extended work the kinetics of the acid hydrolysis of 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylitol have

[109] J. R. Vercellotti, R. Fernandez, and C. J. Chang, *Carbohydrate Res.*, 1967, **5**, 97.
[110] J. R. Vercellotti and E. K. Just, *Carbohydrate Res.*, 1967, **5**, 102.
[111] E. Tomita, Y. Hirota, and Y. Nitta, *J. Pharm. Soc. Japan*, 1967, **87**, 479.
[112] E. Tomita, *J. Pharm. Soc. Japan*, 1967, **87**, 485.
[113] M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, 1967, **5**, 453.

been investigated and discussed in relationship to the role of these compounds in polysaccharide chemistry.[114] The kinetics of the acid hydrolysis of 1,6-linked aldobiouronic acids and their neutral counterparts (disaccharides) have been examined, and the results compared with those obtained in work on 1 → 4 and 1 → 2-linked compounds described previously. In all cases the neutral compounds were hydrolysed more rapidly than the acids and this relationship showed a marked dependence on the inter-saccharide linkage position.[115] In addition, the alkaline hydrolysis of alkyl and aryl β-D-glucopyranosiduronic acids has been examined in detail;[116] electron-withdrawing groups now increased the reactivity of the glycosides. Studies were carried out in different alkalinities and Hammett functions were calculated.[116] Similarly, the acidic and alkaline hydrolysis of the α- and β-anomers of glucofuranosidurono-3,6-lactones have been examined in detail, and comparisons have been made with the rates of reaction of the free acids.[117]

Other features of glycoside-bond cleavage have been investigated. The radical cleavage of glucuronides has been studied using either hydrogen peroxide, in which case a variety of metal ions promote reaction, or ascorbic acid, with which cupric ions act catalytically.[118] The glycosidic bond of methyl α-D-glucopyranoside can be cleaved by γ-radiation from a ^{60}Co source, and it has been reported that aromatic ester functions on the glycoside stabilised the bond.[119a] A variety of substituted benzoates were considered to afford considerable protection, but the analytical procedures used may be considered to be suspect, so doubt is cast on the quantitative information given. Toluene-*p*-sulphonates were less effective protecting groups.[119a]

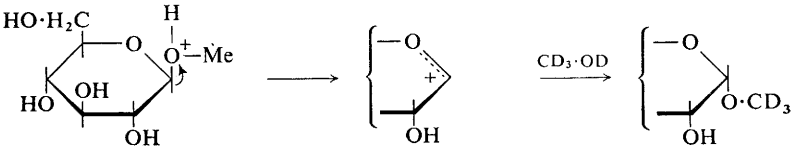
The hydrolysis of the glycoside amygdalin [D(−)mandelonitrile β-gentiobioside] by a preparation of almond emulsin has been shown to proceed with the sequential liberation of prunasin and D-glucose, mandelonitrile and D-glucose, benzaldehyde and hydrogen cyanide.[119b] Gentiobiose was not formed during the hydrolysis, and it was proposed that three different enzymes were involved, each of which specifically catalysed one hydrolytic stage.

The application of dihalomethyl methyl ethers with zinc chloride as reagents for the cleavage of oligosaccharide glycosides has been described. For example, the flavonol glycoside, rutin, as its acetate, gave the *O*-acetylrutinosyl halide which was then converted into hepta-*O*-acetyl rutinose [6-*O*-(α-L-rhamnopyranosyl)-D-glucose] in the usual way.[120] Methyl and

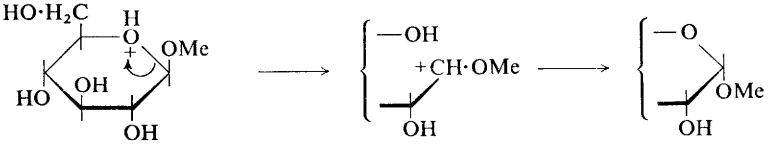
[114] A. Meller, *J. Polymer Sci.*, Part *A*-1, *Polymer Chem.* 1967, **5**, 1443.
[115] K. K. De and T. E. Timell, *Carbohydrate Res.*, 1967, **4**, 177.
[116] E. Tomita, *J. Pharm. Soc. Japan*, 1967, **87**, 490.
[117] E. Tomita and Y. Nitta, *J. Pharm. Soc. Japan.*, 1967, **87**, 495.
[118] Y. Yamane, K. Sakai, and K. Ikeguchi, *J. Pharm. Soc. Japan*, 1967, **87**, 227.
[119a] I. M. Sarkar and J. C. Arthur, *Chem. and Ind.*, 1967, 2085.
[119b] D. R. Haisman and D. J. Knight, *Biochem. J.*, 1967, **103**, 528.
[120] R. Bognár, I. Farkas Szabó, I. Farkas, and H. Gross, *Carbohydrate Res.*, 1967, **5**, 241.

phenyl β-D-cellobiosides were similarly converted to the octa-acetyl-disaccharide in 82 and 49% yield, respectively.[120]

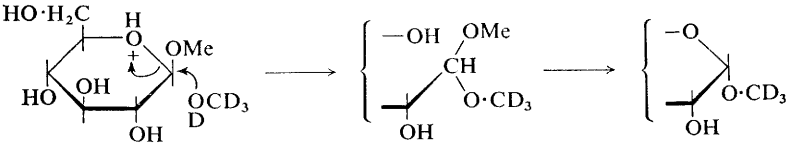
Direct evidence for the detailed mechanism of hydrolysis of glycosides is almost unobtainable, but this is not so for the closely related anomerisation reaction, since characterisation of the source of the aglycone in the initial products allows a distinction to be made between a cyclic carbonium ion process and an acyclic mechanism. Capon and Thacker [121, 122] have now done this by carrying out the anomerisation of methyl α- and β-D-gluco-pyranosides in perdeuteriomethanol, containing methanesulphonic acid as catalyst. The overall reaction and the incorporation of trideuteriomethyl groups could be followed simultaneously by n.m.r. techniques, and it was found that the labelled groups were incorporated from the outset. Of the possible mechanisms (Schemes 2–4) therefore, Scheme 3 can now be



Scheme 2



Scheme 3



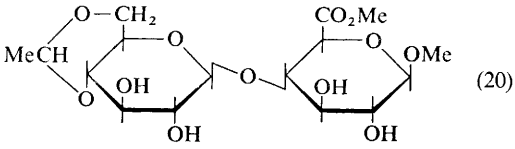
Scheme 4

excluded and Scheme 4 cannot apply since it is known that D-glucose dimethyl acetal reacts to give furanosides under the conditions of the experiment. Displacements at C-1 occurred predominantly with inversion of configuration.

Other Reactions.—Uronic acid-containing disaccharides of polymers can be cleaved by an elimination rather than a hydrolytic process, and it has now been shown that methyl 4′,6′-*O*-ethylidene-β-pseudocellobiuronoside

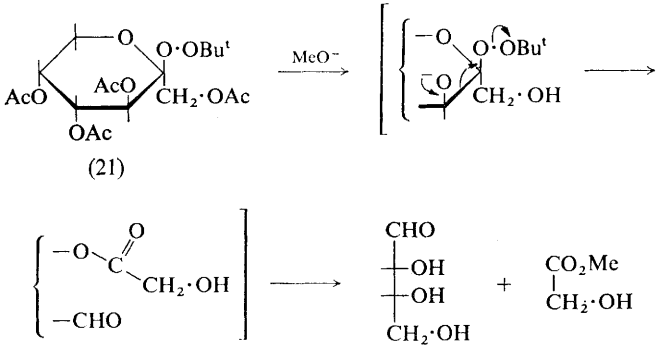
[121] B. Capon, *Chem. Comm.*, 1967, 21.
[122] B. Capon and D. Thacker, *J. Chem. Soc.* (*B*), 1967, 1010.

methyl ester (20) cleaved to give 4,6-*O*-ethylidene-D-glucose on treatment with hydrazine, presumably by such a route. It was suggested,[123] therefore, that 1 → 4-linkages adjacent to the carboxy-groups of uronic acid-containing polysaccharides will be cleaved by this reagent, but it might be expected that this would be so only when the carboxylate ionic form was not present.



(20)

The glycosyl peroxides obtained by treatment of t-butyl hydroperoxide with *O*-acyl-glycosyl halides may be considered as glycoside derivatives. The peracetates of the *β*-D-*gluco*, *β*-D-*galacto*, *α*-D-*manno*, *α*-L-*rhamno*, *β*-D-*xylo*, and *α*-L-*arabino* t-butyl peroxides have now been described together with the *α*-L-arabinofuranosyl analogue. Each, on mild treatment with sodium methoxide in methanol, afforded the corresponding lower aldose (minus C-1) in good yield.[124] Application of the reaction provided a method for reducing a ketose chain by two carbon atoms;[125] thus the crystalline fructopyranosyl derivative (21) gave D-erythrose (97%) as shown in Scheme 5. Similar reaction in the L-sorbose series gave L-threose.



(21)

Scheme 5

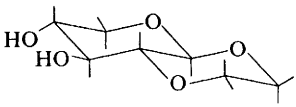
The n.m.r. spectrum of (21) was discussed.[125] Extensions of this work to aldonyl peroxides and to phenylazo-hydroperoxides are outlined on pp. 101 and 144.

The effects of chlorine dioxide on the chlorine oxidation of glycosides have been investigated since it is known that the presence of the oxide decreases the degradation of cellulose during bleaching. Marked reduction

[123] M. Fujinaga and Y. Matsushima, *Bull. Chem. Soc. Japan*, 1967, **40**, 1706.
[124] M. Schulz, H. Boeden, and P. Berlin, *Annalen*, 1967, **703**, 190.
[125] M. Schulz, H. Boeden, and E. Gründemann, *Z. Chem.*, 1967, **7**, 13.

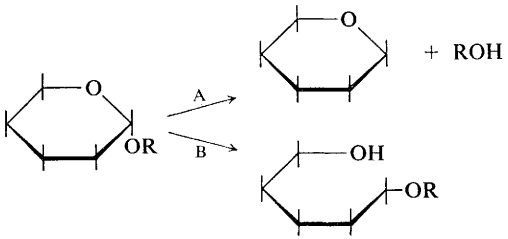
in oxidation rates resulted from the addition of the oxide which was taken to indicate that it is acting as a radical scavenger. The influence of light on the reaction was also assessed.[126]

Treatment of 2-chloroethyl-glycosides with base gave 1,2-*O*-ethylene derivatives; compounds in the β-D-xylose (22) and α-L-arabinose series have now been synthesised.[127]



(22)

A series of 2-alkoxy- and 2-aryloxy-tetrahydropyrans, which serve as model glycosides, and their 6-methyl and 6-hydroxymethyl derivatives have been subjected to hydrogenolysis with lithium aluminium hydride–aluminium chloride in ether solution, and the relative importance of the reaction routes A and B (Scheme 6) have been determined.[128] When

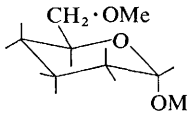


Scheme 6

R = Me products formed by route A predominated (70%); with other primary alkyl derivatives routes A and B had similar significances; when R was a secondary or tertiary alkyl group, route B represented the major reaction path. Aryl derivatives, on the other hand, cleaved by side-chain rupture (route A) exclusively. The results were explained [128] in terms of polar effects, but steric factors were also considered to exert an influence. During the course of this work the conformations of *cis*- and *trans*-2-ethoxy-6-methyltetrahydropyran and 2-methoxy-6-methoxymethyltetrahydropyran were examined by n.m.r. spectroscopy and in each case the chair form having the 6-substituent in the equatorial orientation was preferred. With the latter isomers, the *trans*-form (23), with an axial C-2-methoxy-group, was favoured thermodynamically because of the anomeric effect.

The relative reactivities of the hydroxy-groups in methyl β-D-glucopyranoside under Koenigs–Knorr conditions have been investigated by

[126] P. S. Fredricks, B. O. Lindgren, and O. Theander, *Acta Chem. Scand.*, 1967, **21**, 2895.
[127] G. R. Inglis, *J. Chem. Soc.* (*C*), 1967, 2028.
[128] U. E. Diner and R. K. Brown, *Canad. J. Chem.*, 1967, **45**, 2547.



(23)

using [^{14}C]tetra-*O*-acetyl-D-glucosyl bromide and determining the ratios of disaccharides formed. The order of reactivities was 6-OH$\gg$3-OH$>$4-OH $>$2-OH with average values 6·4 : 1·7 : 1·2 : 1·0. Since the C-4-OH was not markedly less reactive than the other secondary groups it was suggested that the difficulties encountered in the synthesis of 1$\rightarrow$4-linked disaccharides may be due to steric hindrance by the adjacent blocking groups.[129] Reactions were carried out in dioxan in the presence of silver oxide and iodine. Relative reactivities of such hydroxy-groups under other conditions have been mentioned.[70,73b]

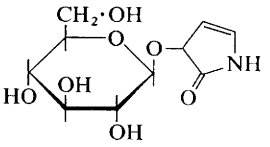
The interesting partial oxidations undergone by methyl glycopyranosides with periodate in DMSO solution are discussed on p. 173.

Natural Products.—Although a wide selection of naturally occurring *O*-glycosides has been discussed in the literature, no attempt is made to include them in this survey unless some particular feature appears to the Reviewers to be of interest to carbohydrate chemists. Reichstein and his co-workers have continued their study of complex plant glycosides and report their findings in a series of detailed papers in *Helvetica Chimica Acta*. Amongst the sugars they found in such compounds were 6-deoxy-D-allose, 6-deoxy-D-glucose, 6-deoxy-L-talose, 6-deoxy-3-*O*-methyl-D-glucose, 6-deoxy-2,3-di-*O*-methyl-D-glucose, 6-deoxy-3-*O*-methyl-D-allose, 2,6-dideoxy-D-*ribo*-hexose, 2,6-dideoxy-3-*O*-methyl-D-*ribo*-hexose, 2,6-dideoxy-3-*O*-methyl-L-*arabino*-hexose, and 2,6-dideoxy-3-*O*-methyl-D-*xylo*-hexose and these frequently as di- and tri-saccharide entities. The mass spectra of 4-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl)-2,6-dideoxy-3-*O*-methyl-L-*arabino*-hexose and its methyl glycoside have been recorded and discussed by these workers.[130]

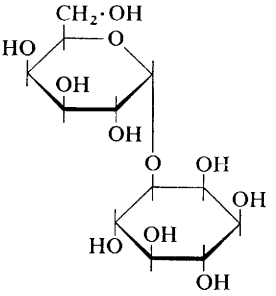
A new crystalline D-glucoside isolated from pea seedlings has been tentatively assigned structure (24) on the basis of chemical, spectroscopic, and enzymic evidence.[131] D-Galactose has been found associated with polyols in several instances: galactinol (25) has been isolated in about 0·1% yield from the leaves of *Lamium maculatum*,[132] 2-*O*-(α-D-galactopyranosyl)-glycerol has been obtained from the red seaweed *Laurencia pinnatifida* and the n.m.r. and mass spectra of its acetate have been recorded.[133] Illumination of euglena which have been grown in the dark

[129] A. M. Bills and J. W. Green, *J. Chem. Soc.* (*B*), 1967, 716.
[130] K. A. Jaeggi, E. Weiss, W. Wehrli, and T. Reichstein, *Helv. Chim. Acta*, 1967, **50**, 1201.
[131] J. Kocourek, V. Bucharová, V. Buchbauerová, V. Jiráček, J. A. Košťíř, J. V. Košťíř, C. Kysilka, I. Mostková, A. Pribylová, M. Tichá, and I. Tobek, *Arch. Biochem. Biophys.* 1967, **121**, 531.
[132] M. Senser and O. Kandler, *Phytochemistry*, 1967, **6**, 1533.
[133] R. T. Aplin, L. D. Durham, Y. Kanazawa, and S. Safe, *J. Chem. Soc.* (*C*), 1967, 1346.

caused, amongst other changes, the appearance of D-galactosyl diglycerides which were believed to serve in the physical arrangement of chlorophyll molecules.[134] L-Rhamnose has been found as a 1 → 4-linked disaccharide

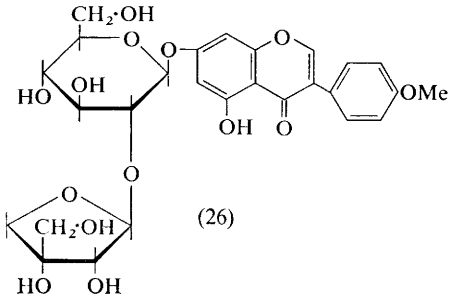


(24)



(25)

attached to a sterol[135] and to (+)-11-hydroxyhexadecanoic acid.[136] Another disaccharide glycoside, lanceolarin (26) isolated from *Dalbergia Lanceolaria*, has been shown to be an isoflavone apiosylglucoside.[137]



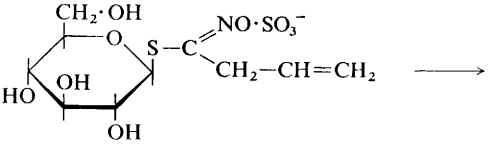
(26)

S-Glycosides

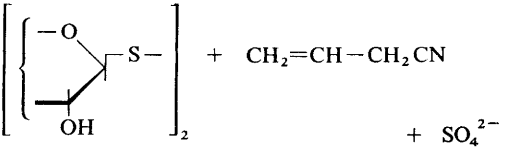
Numerous means for degrading sinigrin (27) and other plant thioglycosides which are toxic to animals have been described, and a new method reported. Ferrous ions were found to catalyse the decomposition as shown, but the degradation did not occur with ethyl 1-thio-β-D-glucopyranoside; a mechanism for a specific reaction was discussed.[138] Other salts also promoted the decomposition, and other thioglycosides found naturally with sinigrin

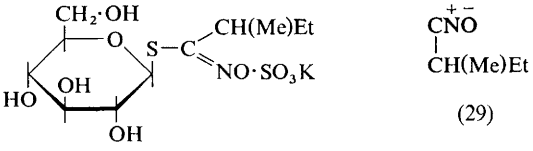
[134] A. Rosenberg, *Science*, 1967, **157**, 1191.
[135] R. V. Krishna Rao and S. Rangaswami, *Tetrahedron Letters*, 1967, 4563.
[136] S. N. Khanna and P. C. Gupta, *Phytochemistry*, 1967, **6**, 735.
[137] A. Malhotra, V. V. S. Murti, and T. R. Seshadri, *Tetrahedron*, 1967, **23**, 405.
[138] C. G. Youngs and A. S. Perlin, *Canad. J. Chem.*, 1967, **45**, 1801.

were also degraded.[138] The related natural glycoside glucocochlearin (28) has been synthesised by condensing 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose with the nitrile oxide (29), followed by sulphonylation and deacetylation.[139]



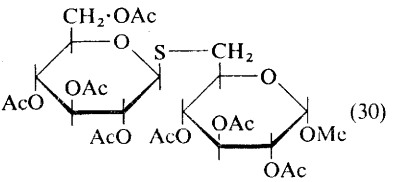
(27)





(28) (29)

The thiodisaccharide 6-*S*-(β-D-glucopyranosyl)-6-thio-D-glucopyranose has been prepared *via* the acetylated glycoside (30) which was obtained by acetylation of the products of condensation either of tetra-*O*-acetyl-D-glucosyl bromide with methyl 6-thio-α-D-glucopyranoside (55%), or of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose with methyl 6-*O*-toluene-*p*-

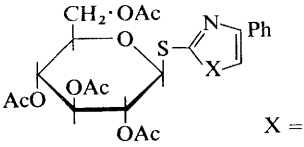


(30)

sulphonyl-α-D-glucopyranoside (19%).[140] The deacetylated glycoside could not be hydrolysed specifically to give the disaccharide in spite of the known relative resistance of thioglycosides to acid; D-glucose and 6-thio-D-glucose were always obtained concurrently. Chromatography on charcoal–Celite allowed the isolation of the required compound.

[139] M. H. Benn and L. Yelland, *Canad. J. Chem.*, 1967, **45**, 1595.
[140] D. H. Hutson, *J. Chem. Soc.* (*C*), 1967, 442.

The synthesis and some reactions of the four possible methyl 1-thio-D-ribosides have been described.[141] The tri-*O*-benzoyl-D-ribofuranosyl and -pyranosyl bromides afforded β-isothiouronium salts on treatment with thiourea in acetone, and these were converted to the corresponding β-thioglycosides by standard procedures. Alternatively, the α-furanosyl compound was prepared by a mercury-catalysed cyclisation of D-ribose dimethyl dithioacetal. Finally the α-pyranoside was obtained by an S_N2 displacement from the β-bromide; the neighbouring benzoate group did not participate therefore in this reaction. The methanethiolysis of D-ribose, in the presence of hydrochloric acid, gave the acyclic acetal as the main product,[141] but furanosides and pyranosides were also formed directly from the sugar. Hydrolysis of the α-pyranoside gave rise to the β-isomer as well as the furanosides, which indicated that the reaction proceeded by an acyclic mechanism, that is, ring-oxygen protonation and ring-opening occurred initially. This did not conform with previous conclusions, nor with the favoured mechanism of hydrolysis of *O*-glycosides.



(31)

X = O or S

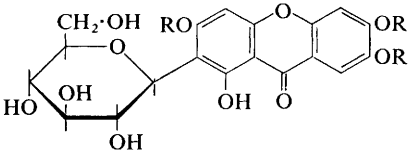
Anomerisation of the glycosides (31) can be brought about in boiling xylene in the presence of mercuric bromide.[142] The syntheses of 2- and 4-(β-D-glucopyranosyl)-selenopyridines have been described, and comparison of their acid hydrolysis with those of the 2- and 4-oxygen and sulphur analogues showed that for each pair the 2-derivative was hydrolysed faster than the 4-isomer and that for each positional series the rates fell in the order O > Se > S.[143]

C-Glycosides

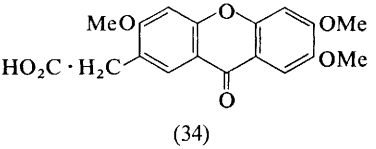
The position of attachment of the glycosyl moiety in the naturally occurring *C*-glycoside, mangiferin (32), previously assigned on the basis of spectroscopic evidence, has now been confirmed by conversion of the trimethyl ether (33) into the acid (34) by alkali fusion.[144] This same product (34) was then prepared from 1-hydroxy-3,6,7-trimethoxyxanthone by way of the 1-allyl ether, which underwent a Claisen rearrangement to give the 2-*C*-allyl derivative, and this was then oxidised to the acetic acid derivative with permanganate and periodate. Mangiferin (32) was obtained synthetically (in poor yield however) from 1,3,6,7-tetrahydroxyxanthone with

[141] C. J. Clayton, N. A. Hughes, and S. A. Saeed, *J. Chem. Soc.* (*C*), 1967, 644.
[142] P. Nuhn and G. Wagner, *Z. Chem.*, 1967, **7**, 154.
[143] G. Wagner and G. Valz, *Pharmazie*, 1967, **22**, 548.
[144] P. E. Nott and J. C. Roberts, *Phytochemistry*, 1967, **6**, 741, 1597.

tetra-*O*-acetyl-D-glucosyl bromide as glycosylating agent and sodium methoxide as base.[144] These last reagents presumably interacted in a competitive reaction which reduced the efficiency of the process.



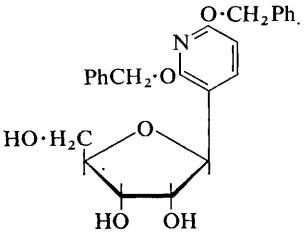
(32) R = H
(33) R = Me



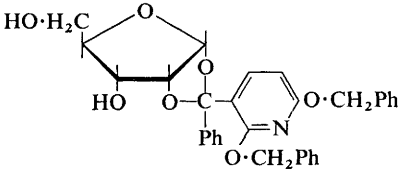
(34)

A compound believed to be a di-*C*-D-glucopyranosylapigenin has been prepared by glucosylation of naringenin.[145] Other known *C*-glycosides have been isolated from the leaves of *Vitis cinerea*: vitexin (8-*C*-β-D-glucopyranosyl-5,7,4′-trihydroxyflavone); iso-vitexin (the 6-isomer); orientin (8-*C*-β-D-glucopyranosyl-5,7,3′,4′-tetrahydroxyflavone); and iso-orientin (the 6-isomer).[146]

The known biological activity of deazanucleosides has stimulated attempts to prepare these compounds synthetically. Such *C*-glycosides occur naturally but their chemical synthesis is not yet well developed. Condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride with bis-(2,6-dibenzyloxypyridyl)3-cadmium, followed by hydrolysis of the ester groups afforded the required 3-(D-ribofuranosyl)-2,6-dibenzyloxypyridine (35) in 10% yield. This on hydrogenolysis gave 1-deazauridine which proved to be chemically unstable.[147] In addition to the *C*-glycoside (35), the acetal (36) was formed in appreciable amounts following nucleophilic attack on a C-1/C-2 benzoxonium ion. Similar synthesis with 3,5-di-*O*-*p*-toluyl-2-deoxy-D-*erythro*-pentofuranosyl chloride afforded,[147] after de-esterification and hydrogenolysis, the 2′-deoxynucleoside analogue of (35)



(35)

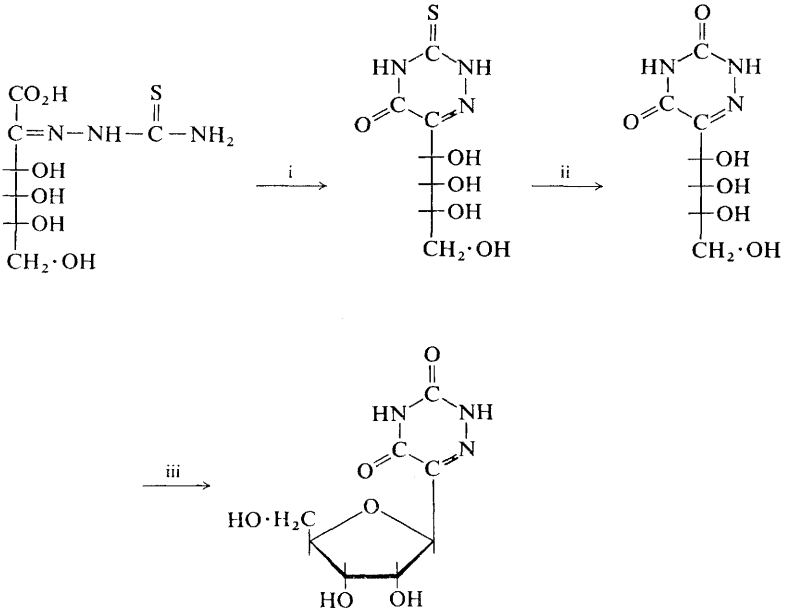


(36)

[145] J. Chopin and M. L. Bouillant, *Compt. rend.*, 1967, **C**, 264, 1875.
[146] H. Wagner, J. Patel, L. Hörhammer, F. Yap, and A. Reichardt, *Z. Naturforsch.*, 1967, **22B**, 988.
[147] M. P. Mertes, J. Zielinski, and C. Pillar, *J. Medicin. Chem.*, 1967, **10**, 320.

in good yield. Again it proved to be chemically unstable presumably, as in the previous case, because of β-eliminations initiated by the carbonyl functions within the heterocycle. No attempts were made to determine the anomeric configurations of the glycosides, but, presumably, compound (35) has the β-structure, as shown, since the C-2-benzoate participated, as evidenced by the identification of compound (36).

Another approach[148] has used the cyclisation of the thiosemicarbazone of a hexulosonic acid to give a base with a polyol side-chain, which under acidic conditions cyclised to give a furanoid ring. An example is shown in Scheme 7.



Reagents: i, 0·5M-NaOH; ii, aq. MeI; iii, 5M-HCl.

Scheme 7

Other Aspects of Glycoside Chemistry

The n.m.r. spectra of aryl glycopyranoside tetra-acetates have been examined in deuteriochloroform with special reference to the anomeric proton resonances. In the α-series these signals were readily observable with $J_{1,2}$ 3–3·5 c./sec.; with the β-compounds they occurred at higher fields and overlapped with those derived from the other ring protons.[149] The o.r.d. spectra of a number of tetrahydropyranyl ethers (perdeoxyglycosides) in which the aglycone was steroidal have been studied. Absolute

[148] M. Bobek, J. Farkaš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3572.
[149] Y. Tsuzuki and K. Tanaka, *Bull. Chem. Soc. Japan*, 1967, **40**, 1208.

configurations have been assigned to the 'anomeric' positions by comparison with curves derived from 2-deoxyglycosides of known configuration; rotational information was given on a number of such glycosides.[150] To examine the potential of mass spectrometry for structural studies of naturally occurring aroyl and aryl glycosides, a number of such compounds and related derivatives have been investigated as their peracetates. It was shown that the identity of the aroyl group can be determined and that the fragmentation pattern of 6-aroylated aryl glycosides was definitive.[151]

Hydrogen-bonding in crystalline methyl β-D-xylopyranoside and β-D-glucopyranose has been investigated by i.r. spectroscopy; no unbonded hydroxy-groups could be detected which contradicts evidence obtained from crystallographic analyses. It was concluded that the supposedly free groups are weakly hydrogen-bonded.[152] Ingenious application of g.l.c. methods has allowed the stereochemistry of the enzymolysis of glucosides to be determined. The first products of cleavage were trimethylsilylated before analysis, and thus it was shown that α- and β-*exo*-glucanases hydrolyse glycosidic linkages with inversion, whereas α- and β-glucosidases hydrolyse with retention of anomeric configuration.[153] The chromatographic characteristics of cardiac glycosides of various sugars have been discussed in terms of conformational analysis and intramolecular interactions.[154]

[150] W. Klyne, W. P. Mose, P. M. Scopes, G. M. Holder, and W. B. Whalley, *J. Chem. Soc.* (*C*), 1967, 1273.
[151] E. Haslam, *Carbohydrate Res.*, 1967, **5**, 161.
[152] A. J. Michell, *Carbohydrate Res.*, 1967, **5**, 229.
[153] F. W. Parrish and E. T. Reese, *Carbohydrate Res.*, 1967, **4**, 424.
[154] L. Nover, G. Baumgarten, and M. Luckner, *J. Chromatog.*, 1967, **32**, 141.

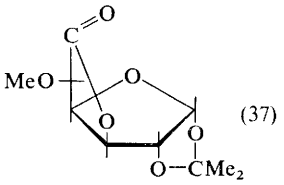
4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—Several syntheses of simple ethers of sugars have been reported and they have been used extensively in g.l.c. and in mass spectrometry studies.

The naturally-occurring sugar javose (6-deoxy-2-*O*-methyl-D-allose) has been synthesised from methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside. Oxidation at C-3 followed by reduction permitted entry into the D-allose series, and standard conversions then afforded the natural compound, the structure of which was therefore confirmed.[155] Reichstein and his co-workers have described the occurrence of many related ethers in *Helvetica Chemica Acta.*

2-*O*-Methyl-D-mannose has been synthesised, the key step being methylation of 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose using diazomethane and boron trifluoride etherate, which was recommended as a methylating reagent applicable to compounds which contain base-labile substitutes;[156] alternative preparations of 1-, 2-, and 3-*O*-monomethyl-D-mannitols have been reported.[157] 4-*O*-Methyl-D-arabinose has been synthesised by a new route from 1,2-*O*-isopropylidene-5-*O*-methyl-D-glucuronolactone (37)



(37)

which was reduced to 1,2-*O*-isopropylidene-5-*O*-methyl-α-D-glucofuranose. Acetylation and hydrolysis gave 3,6-di-*O*-acetyl-5-*O*-methyl-D-glucose which was cleaved with periodate to give the 2,5-diacetate of 4-*O*-methyl-D-arabinose from which the required ether was obtained by catalytic de-esterification.[158] The preparation of 2,4-di-*O*-methyl-D-galactose and

[155] J. S. Brimacombe and A. Husain, *J. Chem. Soc.* (*C*), 1967, 1503.
[156] J. O. Deferrari, E. G. Gros, and I. O. Mastronardi, *Carbohydrate Res.*, 1967, **4**, 432.
[157] S. Bayne, J. A. Fewster, A. J. Grieve, and M. L. Hawksley, *J. Chem. Soc.* (*C*), 1967, 114.
[158] S. C. Williams and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 275.

its 6-deoxy-derivative has been described;[159] methylation of methyl 3,6-di-*O*-methanesulphonyl-β-D-galactopyranoside afforded the 2,4-di-*O*-methyl derivative, which on reduction with lithium aluminium hydride in anhydrous THF gave a mixture of methyl 2,4-di-*O*-methyl-β-D-galactopyranoside and the corresponding 3,6-anhydride. However, when the reduction was carried out in ether–benzene, methyl 6-deoxy-2,4-di-*O*-methyl-β-D-galactopyranoside was obtained in 60% yield. Hydrolysis of the two di-*O*-methyl glycosides afforded the hexoses; the 6-deoxy-compound is a constituent of the antibiotic, labilomycin. Other methyl ethers of D-galactose, including the previously undescribed 2,5-diether, have been prepared by similar means from methyl 6-*O*-trityl-β-D-galactofuranoside.[160]

G.l.c. has been assessed as a means for characterising the methyl ethers of D-xylose which are encountered in structural work on polysaccharides.[161] The mobilities of the acetylated derivatives of the polyols derived from all the ethers except 5-*O*-methyl-D-xylose were recorded. As a method for characterising sugars, this procedure suffered from the disadvantage that, for example, 2-*O*-methyl-D-xylose and the 4-isomer gave unresolvable derivatives, and to try to overcome this difficulty, the authors examined the acetylated diethyl dithioacetals. Although with these, the main products obtained from the various sugars were in most cases resolvable, discrete compounds were not formed. The acetylated nitriles of the sugars, prepared by acetylation and dehydration of the oximes were found to be highly suited to the purpose, discrete products being formed which were, in general, separable from each other. It was concluded that all the ethers examined can be identified exclusively by g.l.c. means.[161]

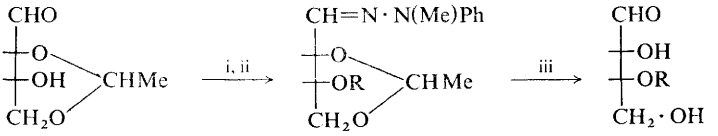
In similar, but more extensive work, a full survey of the methods available for separating and identifying the methyl ethers of arabinose and their derivatives has been reported (74 references recorded).[158] Gas chromatographic studies were undertaken of the ethers themselves, the acetates of the methylated arabinitols, and the acetates of the methyl glycosides. Paper chromatography and t.l.c. were applied to the separation of the free sugars and the glycosides, and while the paper technique was found to be better for separating the free sugars, t.l.c. can provide evidence for the degree of substitution and ring form of the glycosides. Examination of the optical rotations of the compounds revealed that for free L-sugars a specific rotation of +125 to +140° indicated that position 4 was methylated (pyranose ring form), a value of −25° to −50° indicated that the sugar could adopt only the furanose form, and rotations of +90° to +110° indicated that positions 4 and 5 were both unsubstituted. Similar deductions could be drawn from rotations of glycosides.

By application of isotopic techniques and mass spectrometry, Russian workers have developed a method to characterise the position of substitution

[159] J. H. Westwood, R. C. Chalk, D. H. Ball, and L. Long, jun., *J. Org. Chem.*, 1967, **32**, 1643.
[160] I. R. Siddiqui and B. Urbas, *Carbohydrate Res.*, 1967, **5**, 210.
[161] D. G. Lance and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 1995.

of partially methylated sugars.[162] Conversion of the sugars to methyl glycosides and exhaustive methylation with trideuteriomethyl iodide gave compounds, the mass spectra of which depended upon the position of the labelled methyl groups. Distinctive reference spectra for the pentopyranoses, for example, were measured using methyl arabinopyranosides carrying labelled methyl groups at positions 2 or 3, or 3,4.[162] The same approach was adopted in the study of methyl L-arabinofuranosides (labelled at positions 2 or 5, or 2,3, 5 simultaneously);[163] methyl D-galactopyranosides (labelled at 3,6, or 4,6;[164] methyl D-fucopyranosides (labelled at 4, 2 or 3,4).[165]

Substituted Alkyl Ethers.—Benzyl ethers have been used frequently in standard synthetic procedures, usually for the purpose of protecting particular hydroxy-groups, for example, in a new synthesis of ketose derivatives (see p. 175). Two cases of a specific synthesis have been recorded: 3-*O*-benzyl-D-erythrose has been prepared as shown in Scheme 8,[166] and



R=CH_2Ph

Reagents: i, $PhN(Me)NH_2$; ii, $PhCH_2Br$–DMF–Ag_2O; iii, MeCHO–H^+

Scheme 8

1-*O*-benzyl-L-glycerol has been obtained from D-mannitol by way of its 1,6-di-*O*-benzyl-2,5-*O*-methylene derivative.[167]

The synthesis of a 2′,3′,5′-tri-*O*-trityl-ribonucleoside, the uridine derivative, has been recorded,[168] and the trityl group has been employed in numerous standard synthetic procedures. Displacement of a trityl group and glycosylation at the previously protected position during a Koenigs–Knorr reaction has already been described,[68] and the previously recorded observation that trityl ethers can be cleaved on chromatographic silica gel has been utilised in the detritylation of methyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside and 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose.[169] Benzene solutions of the compounds were allowed to percolate on to a column of the adsorbent, where they were left for 16 hr. before

[162] O. S. Chizhov, B. M. Zolotarev, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 277.
[163] O. S. Chizhov and N. F. Madudina, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 284.
[164] N. K. Kochetkov, O. S. Chizhov, and B. M. Zolotarev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 537.
[165] N. K. Kochetkov, O. S. Chizhov, B. M. Kolotarev, and S. H. Sheinker, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 543.
[166] J. W. Van Cleve and C. E. Rist, *Carbohydrate Res.*, 1967, **4**, 91.
[167] J. Gigg and R. Gigg, *J. Chem. Soc.* (*C*), 1967, 1865.
[168] H. U. Blank and W. Pfleiderer, *Tetrahedron Letters*, 1967, 869.
[169] J. Lehrfeld, *J. Org. Chem.*, 1967, **32**, 2544.

elution with ethyl acetate. The activities of four commercial samples of silica gel for detritylation were assessed. A spectrophotometric assay of trityl groups has been developed.[170]

In the search for polymerisable derivatives of carbohydrates 6-*O*-allyl and 6-*O*-methacroyl derivatives of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose have been prepared, the former by treatment of the alcoholic compound with allyl bromide and sodium hydroxide, and the latter by a transesterification reaction of methyl methacrylate with the aid of tetra-isopropyl titanate.[171]

Additional methods have been described for the removal of the useful allyl ether blocking group. Isomerisation under the usual alkaline conditions gave *cis*-prop-1-enyl ethers which were then treated with mercuric oxide and mercuric chloride in aqueous acetone in a transvinylation-type reaction. The procedure was particularly useful for the removal of allyl aglycones in the presence of acid-labile substitutes as, for example, in the preparation of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-galactose from the allyl glycoside.[172]

Unsaturated compounds were also employed in the synthesis of the 2-, 3-, and 6-*O*-methanesulphonylethyl-D-glucoses which were required for comparison with hydrolysis products of substituted celluloses.[173] These were prepared from appropriately substituted glucoses by treatment with methyl vinyl sulphone in dioxan in the presence of sodium phenoxide, followed by removal of the protecting groups. The amorphous products were examined by g.l.c. of the TMS derivatives and were shown to give two peaks corresponding to the α- and β-anomeric forms. In the preparation of the 2-substituted derivatives the allyl protecting group mentioned above was employed.

For similar purposes, and by equivalent means, the 2-, 3-, and 6-*O*-(2-diethylaminoethyl)-D-glucoses were prepared by use of 2-diethylaminoethyl chloride and potassium hydroxide, followed by hydrolysis of the protecting groups. The relative distributions of the 2-diethylaminoethyl groups in the substituted cellulose were examined by comparing the polymer hydrolysate with the authentic monomer derivatives by g.l.c. of the TMS derivatives.[174] In related work the same authors investigated the preferential reactivities of the hydroxy-groups of methyl 4,6-*O*-benzylidene-D-glucopyranosides with 2-diethylaminoethyl chloride (1·1 mol.) in aqueous sodium hydroxide and obtained the results shown in Table 1.[175] Change of the reaction medium to dioxan had little effect on the reactivity of the β-compound, but for the α, 93% of the monosubstitution then occurred at position 3.[176]

[170] R. M. Saunders, H. P. Schwarz, and J. C. Stewart, *Analyt. Chem.*, 1967, **39**, 550.
[171] W. A. P. Black, J. A. Colquhoun, and E. T. Dewar, *Carbohydrate Res.*, 1967, **5**, 362.
[172] R. Gigg and C. D. Warren, *Tetrahedron Letters*, 1967, 1683.
[173] A. L. Bullock, V. O. Cirino, and S. P. Rowland, *Canad. J. Chem.*, 1967, **45**, 255.
[174] E. J. Roberts and S. P. Rowland, *Canad. J. Chem.*, 1967, **45**, 261.
[175] E. J. Roberts and S. P. Rowland, *Carbohydrate Res.*, 1967, **4**, 509.
[176] E. J. Roberts and S. P. Rowland, *Carbohydrate Res.*, 1967, **5**, 1.

Table 1

	2-Ether	*3-Ether*	*2,3-Diether*	*Starting material*
α-Glycoside (%)	6	12	19	59
β-Glycoside (%)	7	12	36	47

Trimethylsilyl Ethers.—These are mentioned many times in this report in connection with their value in g.l.c. analytical work (see especially p. 191). For g.l.c. reference purposes a number of partially trimethylsilylated derivatives of D-glucose have been prepared.[177] Trimethylsilyl tri-*O*-trimethylsilyl-αβ-glucopyranosides with free hydroxy-groups at C-2, C-3, C-4, or C-6 were prepared from the appropriate monobenzyl ethers. Hydrolysis of benzyl tetrakis-*O*-trimethylsilyl-αβ-D-glucopyranoside gave a mixture which consisted largely of trimethylsilyl tetrakis-*O*-trimethylsilyl-α- and -β-glucosides formed as a result of intermolecular migration of the ether groupings. Trimethylsilylation of α- and β-D-glucose with limited amounts of reagent gave trimethylsilyl tetrakis-*O*-trimethylsilyl-D-glucosides together with the 2,3,6- and 2,4,6-tri-*O*-substituted derivatives as the main products.

Fully trimethylsilylated derivatives of xylitol, 1,4-anhydro-xylitol, 2,4-*O*-methylene-xylitol, and 1,4-anhydro-3,5-*O*-methylene-xylitol were reported as having melting points within the range -57 to $+15°$. I.r. and viscometric studies were carried out on the compounds.[178]

The mass spectrometry of trimethylsilyl ethers has been examined. Derivatives of free sugars and glycosides were studied and it was concluded that differences in fragmentation patterns of different sugars were more pronounced than with the corresponding methyl ethers. This method should have great potential in oligosaccharide structural determinations particularly as mass spectrometry can be used in combination with g.l.c.[179] The mass spectra of TMS derivatives of aldonolactones have also been investigated and the technique has been shown to be applicable to molecular weight and ring-size determinations.[180] Diasteroisomers were found to give readily distinguishable fragmentation patterns although the same ions were detected in each case, thus offering an additional method for characterisation of compounds in this series, provided appropriate reference spectra are available.

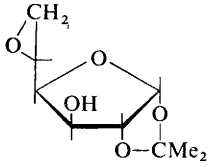
Intramolecular Ethers (Anhydro-sugars)

Epoxides.—The ring-opening of epoxides continues to be an important synthetic reaction in carbohydrate chemistry; this is illustrated by the

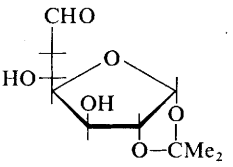
[177] S. M. Kim, R. Bentley, and C. C. Sweeney, *Carbohydrate Res.*, 1967, **5**, 373.
[178] L. A. Nakhapetyan and G. V. Varvanina, *Zhur. obshchei khim.*, 1967, **37**, 395.
[179] O. S. Chizhov, N. V. Molodtsov, and N. K. Kochetkov, *Carbohydrate Res.*, 1967, **4**, 273.
[180] G. Petersson, O. Samuelson, K. Anjou, and E. von Sydow, *Acta Chem. Scand.*, 1967, **21**, 1251.

selection of varied applications reported in 1967. (Cleavage of epoxides of cyclopentane is referred to on p. 156.)

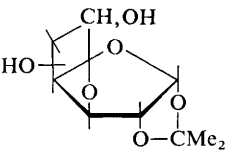
Two procedures have led to new carbon–carbon bonds. The oxo-reaction which has been applied extensively to unsaturated carbohydrates has been extended to epoxides; 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose (38) with carbon monoxide and hydrogen in benzene solution and in the



(38)



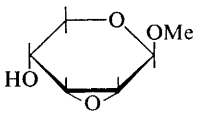
(39)



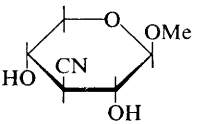
(40)

presence of dicobaltoctacarbonyl gave the bicyclic hemiacetal (40) in 78% yield, presumably formed *via* the deoxydialdose derivative (39).[181] Extension of the procedure to 2,3-epoxides afforded branched-chain sugar derivatives. Details of these last reactions were, however, not reported.[181]

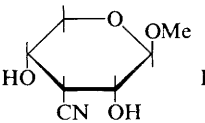
Ring-opening with cyanide ion gave unexpected products. Methyl 2,3-anhydro-β-D-ribopyranoside (41) with aqueous sodium cyanide at pH 8–8·5 and 100° gave a mixture of products from which two isomeric



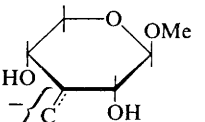
(41)



(42)



(43)



(44)

cyanodeoxy-sugars (42) and (43) were isolated (40% yield); both were shown to be 3-cyano-3-deoxy-glycosides.[182] The D-*ribo*-product (43), with the unexpected 2,3-*cis*-configuration, was shown to arise, not from direct ring-opening, but from (42) by a base-catalysed epimerisation through the anion (44). Compounds (42) and (43) gave the same equilibrated mixture when either was subjected to the reaction conditions, or if heated in aqueous sodium hydrogen carbonate. Cyanide treatment of methyl 2,3-anhydro-α-D-ribofuranoside, however, occurred without apparent complication and by preferential attack at C-3.[183]

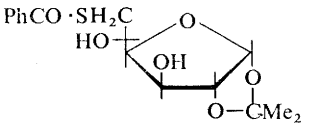
Other work with methyl 2,3-anhydro-pentopyranosides has dealt with the direction of ring-opening of a variety of isomers bearing a 4-azido-4-deoxy-group.[184] Hydroxide ion attacked the α-*lyxo*-enantiomers preferentially at C-3 (*xylo* : *arabino* products, 1 : 4), but the β-*lyxo*-compound

[181] A. Rosenthal and G. Kan, *Tetrahedron Letters*, 1967, 477.
[182] N. R. Williams, *Chem. Comm.*, 1967, 1012.
[183] P. W. Austin, J. G. Buchanan, and R. M. Saunders, *J. Chem. Soc.* (*C*), 1967, 372.
[184] A. J. Dick and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 2879.

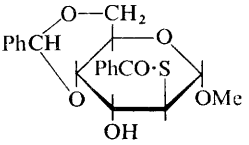
at C-2. With the *ribo*-derivatives, attack occurred predominantly at C-3 in the case of the β-compound (cf. the reaction with cyanide ion above), but little preference was shown with the α-isomer. Related studies were carried out using methoxide ion as the nucleophile. The results were discussed, and a variety of methanesulphonyl esters of the reaction products described.

Epoxide ring-opening with potassium hydrogen fluoride in ethane-1,2-diol provided a satisfactory method for preparing fluorohydrins (p. 85), and with *NN*-dimethylchloroforminium chloride, vicinal dichloro-dideoxy-compounds were formed.[185]

Pyridinium thiobenzoate (obtained simply by taking equimolar quantities of pyridine and thiobenzoic acid) opened epoxides satisfactorily to give thio-derivatives. The epoxide (38), for example, gave the 6-thio-glucose



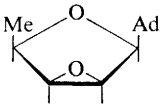
(45)



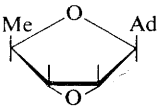
(46)

product (45), and methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside gave, as would be expected, the *altro*-compound (46) as the main product.[186]

Other work with sulphur nucleophiles concerned the ring-opening of the nucleosides (47) and (48) with sodium benzyl sulphide, and the results were compared with those obtained when azide ion was used.[187] With the



(47)



(48)

lyxo-nucleoside (47) the nucleophile attacked with slight preference for the 3′ position, that is the *arabino*-product predominated; with azide ion the preference was more marked and the *arabino* : *xylo* ratio was 3 : 1. Reductive desulphurisation of the thio-compounds afforded the corresponding 2′,5′- and 3′,5′-dideoxy-nucleosides, and the corresponding amino-deoxy-compounds were obtained by hydrogenation of the azido-products. The mercaptide ion reacted specifically at the 3′-position in the case of the *ribo*-nucleoside (48), but azide ion gave a ratio of the *xylo* :

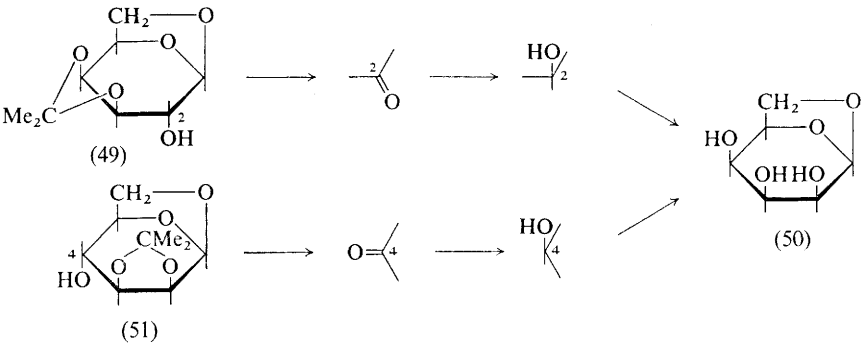
[185] S. Hanessian and N. R. Plessas, *Chem. Comm.*, 1967, 1152.
[186] J. Kocourek, *Carbohydrate Res.*, 1967, **3**, 502.
[187] E. J. Reist, D. F. Calkins, and L. Goodman, *J. Org. Chem.*, 1967, **32**, 2538

arabino products of 4 : 1, together with a third product believed to be the 3,3′-anhydro-nucleoside. Reduction as before gave the appropriate deoxy- and amino-derivatives. The results were discussed and rationalised mainly on steric grounds. It may be useful to note that isomers having free C-2 hydroxy-groups were eluted from anion resin columns more slowly than those carrying substituents at this position.

Treatment of epoxides with potassium selenocyanate gave mainly olefins although α-hydroxy-selenocyanates could be formed (this reaction is dealt with more fully on p. 129). Ring-opening of epoxides having adjacent *trans-O*-acetyl groups with boron trifluoride was shown to involve acetoxy-participation, since reduction of the intermediates gave rise to cyclic acetals (see p. 50).

Despite a report to the contrary,[188] a smooth synthesis of 1,2:5,6-dianhydro-D-mannitol has been reported to occur when 1,6-dibromo-1,6-dideoxy- or 1,6-di-*O*-methanesulphonyl-D-mannitol is treated with aqueous sodium hydroxide with the pH held at 8. The product is a powerful inhibitor of Walker rat carcinoma.[189]

Other Anhydrides*.—The remaining unknown 1,6-anhydro-α-D-hexopyranose, 1,6-anhydro-β-D-talopyranose (50) has been synthesised by the two routes shown below,[190,191] starting from 1,6-anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose (49) or 1,6-anhydro-2,3-*O*-isopropylidene-β-

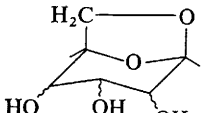


D-mannopyranose (51). Reduction of the intermediate ketones with hydrogen over palladium did not show the high stereoselectivity of complex metal hydrides. Both ketones showed the expected negative Cotton effects and circular dichroism ellipticity in chloroform solution.[190,191] The completion of the 1,6-anhydropyranose series allowed a full study of the optical

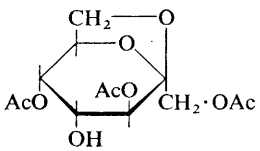
[188] A. Dávid, G. Horváth, I. P. Horváth, L. Institóris, A. Neszmélyi, and L. Radics, *Experientia*, 1967, **23**, 512.
[189] M. Jarman and W. C. J. Ross, *Chem. and Ind.*, 1967, 1789.
[190] D. Horton and J. S. Jewell, *Carbohydrate Res.*, 1967, **5**, 149.
[191] K. Heyns, J. Weyer, and H. Paulsen, *Chem. Ber.*, 1967, **100**, 2317.

* Anhydro-sugars resulting from deamination of amino-sugars are discussed in Section 8.

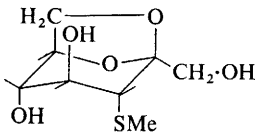
activity of these compounds to be made. Expression of the rotation of the eight isomers (and their 2,3,4-triacetates) in terms of contributions from five constitutive asymmetric elements (after the method of Whiffen) gave self-consistent results which suggested that each of the compounds adopts a conformation close to the regular 1*C* chair form (52).[192] The platinum-catalysed oxidations of these anhydrides and the o.r.d. spectra of the ketonic products are described on pp. 174 and 190: the opening of the anhydro-ring of 1,6-anhydroglucose with hypophosphorus acid is mentioned on p. 149.



(52)



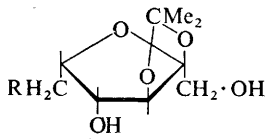
(53)



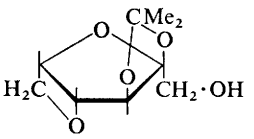
(54)

The acetylation of 2,7-anhydro-β-D-*altro*-heptulopyranose ('sedoheptulosan') which has the same ring structure as (52), has previously been shown to give a small amount of a product (53) with one unreacted hydroxy-group. The yield of this product has now been improved and its structure elucidated by oxidation of the free hydroxy-group followed by reduction and simultaneous deacetylation to a mixture of anhydrides shown to be 2,7-anhydro-β-D-*altro*- and -*manno*-heptulopyranoses.[193] The free hydroxy-group was consequently at C-4. In agreement with this, the ester obtained by sulphonylation and deacetylation did not reduce sodium periodate nor lead tetra-acetate. A 3,4-epoxide obtained on treatment of the toluene-*p*-sulphonate with sodium methoxide gave an epoxide which cleaved as expected to give the *trans*-diaxial product (54) when treated with sodium methyl sulphide. Desulphurisation of (54) gave a deoxy-compound which was resistant to oxidation by periodate, but which could be cleaved with lead tetra-acetate in pyridine, a behaviour consistent with the presence of vicinal *trans*-diaxial hydroxy-groups on an anhydropyranose ring.[193]

The anhydroketose derivative (56) was obtained by treatment of the 6-*O*-toluene-*p*-sulphonyl or the 6-deoxy-6-iodo-derivative (55) of 2,3-*O*-isopropylidene-α-L-*xylo*-hexulofuranose with silver fluoride in pyridine.[194]



(55) R = Ts or I



(56)

[192] D. Horton and J. D. Wander, *J. Org. Chem.*, 1967, **32**, 3780.
[193] E. Zissis, *J. Org. Chem.*, 1967, **32**, 660.
[194] L. Hough and B. A. Otter, *Carbohydrate Res.*, 1967, **4**, 126.

The 1-*O*-toluene-*p*-sulphonyl derivative of (56) was shown to be identical with a compound described in the literature as '1,4-anhydro-2,3-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-L-*xylo*-hexulofuranose'. Other workers have prepared this compound and several analogues of (55) and (56) in closely related work.[195]

In a study of the ring-opening reactions of 3,6-anhydroglycosides, Foster and co-workers [196] have shown that the action of boron trichloride on 3,6-anhydroglucosides is complex. The methyl α-pyranoside gave, after benzoylation of the product mixture, methyl 2,3,4-tri-*O*-benzoyl-6-chloro-6-deoxy-α-D-glucoside, an isomer of this and, as the main product, an amorphous, methoxy-free compound which analysed for a tetra-*O*-benzoyl-chloro-deoxy-hexose. Reaction of the α-furanoside was even more complex. After benzoylation, the products isolated were the dibenzoates of the starting material and of its β-anomer, the isomer of the new glycoside found above, together with a methoxy-free, chloro-compound as the main product. Clearly glycoside cleavage occurred concurrently with anhydro-ring opening.

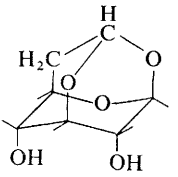
Anhydrides can be formed under unexpectedly mild conditions as, for example, during the mono-*O*-toluene-*p*-sulphonylation of 2,3,5-tri-*O*-benzyl-D-arabinitol which resulted in the formation of 1,4-anhydro-2,3,5-tri-*O*-benzyl-D-arabinitol.[197]

Details have been published of the optimum conditions for the catalytic reduction of acylglycosyl halides to 1,5-anhydroalditol esters, and for the production of 2-deoxy- and 2,3-dideoxy-derivatives from glycal and hydroxyglycal esters.[198] Barker and his co-workers have also investigated [199] the synthesis of five-membered anhydropolyols by acid-catalysed dehydrations of the parent alditols. Kinetic measurements lead to the conclusion that cyclisation involved displacement of protonated primary hydroxy-groups by nucleophilic hydroxy-groups. The rates of the reactions were strongly dependent upon the inductive influence of groups adjacent to the interacting functions and to configurations at all asymmetric centres. Each of these effects was discussed in detail.[199]

[195] O. Fehér and L. Vargha, *Kémiai Közlemények*, 1967, **28**, 343.
[196] M. A. Bukhari, A. B. Foster, and J. M. Webber, *Carbohydrate Res.*, 1967, **4**, 105.
[197] Y. Rabinsohn and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 3452.
[198] G. G. Gray and R. Barker, *J. Org. Chem.*, 1967, **32**, 2764.
[199] B. G. Hudson and R. Barker, *J. Org. Chem.*, 1967, **32**, 3650.

5
Acetals

This report will be subdivided according to whether carbohydrate carbonyl or hydroxy-groups are involved. One interesting example of an acetal (57) formed intramolecularly between a carbonyl and two hydroxy-groups has been reported;[200] its n.m.r. spectral features are described on p. 184.



(57)

Acetals Derived from Carbohydrate Carbonyl Groups

Complete acetolysis of the acetylated ethylene dithioacetals of *aldehydo*-D-glucose, -D-mannose, and -L-arabinose gave the corresponding *aldehydo*-aldose peracetates, but a complex mixture of products was obtained from the D-galactose derivative.[201] Other workers have acetolysed one of the acetal bonds specifically, and have shown that peracetates of aldose acetals and thioacetals gave initial monosubstituted products which then epimerised.[202] Rates of these reactions were shown to fall in the order:

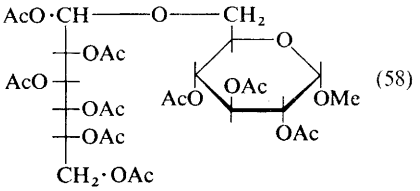
$$\underset{\text{(epimerisation)}}{RS\cdot\dot{C}H\cdot OAc} > \underset{\text{(acetolysis)}}{\cdot CH(OR)_2} > \underset{\text{(acetolysis)}}{\cdot CH(SR)_2}$$

In addition it was shown [202] that such substitutions occurred faster than when the corresponding leaving groups were attached at C-1 in peracetyl glycopyranosyl derivatives. Acetolysis of peracetylaldose dialkyl acetals afforded a means for preparing peracetyl-aldose monoalkyl acetals. The same authors [203] have measured the rate constants for the equilibration and for C-1-acetoxy-exchange reactions of the diastereoisomeric 1,2,3,4,5-penta-*O*-acetyl-L-arabinose *S*-ethyl monothioacetals and have shown that

[200] J. C. Jochims, G. Taigel, and W. Meyer zu Reckendorf, *Tetrahedron Letters*, 1967, 3227.
[201] J. Fernandez-Bolanos and R. Guzman de Fernandez-Bolanos, *Anales real Soc. espaň. Fis. Quim.*, 1967, **63**, 487.
[202] N. H. Kurihara and E. P. Painter, *Canad. J. Chem.*, 1967, **45**, 1467.
[203] E. P. Painter and N. H. Kurihara, *Canad. J. Chem.*, 1967, **45**, 1475.

the C-2-acetoxy-group did not participate, the rate-determining step in the reaction was the direct formation of the C-1 carbonium-sulphonium ion. Extensive discussion of neighbouring-group participation by acetoxy-groups was given, and reactions of several acetal-like derivatives were discussed.

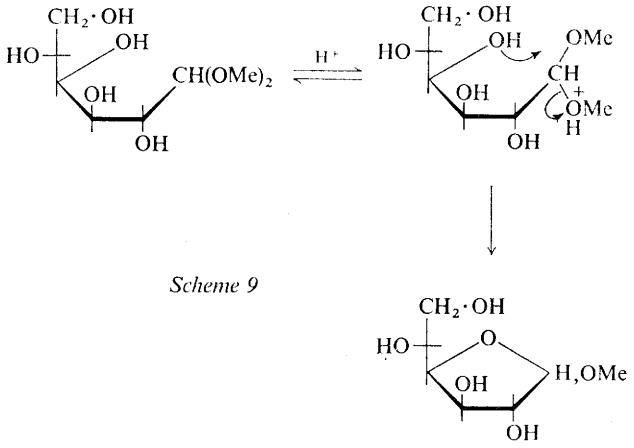
A new type of disaccharide with a monoacetal structure has resulted from treatment of penta-*O*-acetyl-*aldehydo*-D-glucose with, for example, methyl α-D-glucopyranoside in acetic anhydride–pyridine. In this case the product was believed to have structure (58) but neither with this compound



(58)

nor with others obtained using methyl α-D-galactopyranoside, or the 6-*O*-trityl or 4,6-*O*-benzylidene derivatives of methyl α-D-glucopyranoside, was the structure rigorously established.[204]

The hydrolysis of the dimethyl acetals of D-glucose, D-galactose, and L-arabinose has been examined in detail in dilute aqueous acid and it was established that furanoside formation occurred concurrently. Since the rates of ring-closure substantially exceeded those expected, it was concluded that they proceeded by a concerted process in which the oxygen at C-4 assisted the rupture of the acetal bond as shown for the D-*gluco*-example (Scheme 9).[205] A range of rate constants for the hydrolysis reaction of



Scheme 9

[204] F. Micheel and H. J. Stimbers, *Carbohydrate Res.*, 1967, **5**, 218.
[205] B. Capon and D. Thacker, *J. Chem. Soc.* (*B*), 1967, 1322.

furanosides and acetals were given and were discussed, and the n.m.r. chemical shifts of the C-1 protons and of the methoxy-protons of the acetal of D-galactose and the four methyl glycosides were recorded.[205]

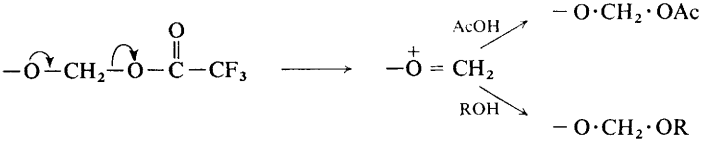
Condensation of D-xylose with methanol–benzaldehyde mixtures in the presence of acid catalysts is known to give the 2,4:3,5-di-*O*-benzylidene derivative of D-xylose dimethyl acetal in high yield. The same reaction has now been shown to occur with other benzaldehyde–primary alcohol mixtures to give analogous products all of which gave the acetals on hydrogenolytic removal of the benzylidene rings. However, when secondary alcohols were employed 1,2:3,5-di-*O*-benzylidene-D-xylofuranoses were the main products.[206] These and the isopropylidene derivatives formed from D-xylose dimethyl and diethyl dithioacetal are described under the following heading.

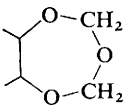
Acetals Derived from Carbohydrate Hydroxy-groups

From Diols on Acyclic Carbohydrates.—The di-*O*-isopropylidene derivative obtained on acetonation of D-xylose diethyl dithioacetal with an acidic resin as catalyst has been shown to have the 2,3:4,5 rather than the previously assigned 2,4:3,5 structure.[207] The products of monoacetonation were shown to be the 4,5-, 3,4-, 2,3-, and 2,4-derivatives, and isolation of these compounds afforded new routes to 2,3- and 2,5-di-*O*-methyl-D-xylose. Methanolysis of the former dimethyl ether, in the presence of acidic resin, gave the α-furanoside in crystalline form.[207] In closely similar work the analogous reaction with D-xylose dimethyl dithioacetal also gave the 2,3:4,5 product when sulphuric acid was used as catalyst, but when zinc chloride or copper sulphate were employed 3,4-, 4,5-, and 2,3:4,5- derivatives were obtained together with another fully substituted compound.[208] Brief treatment of the 4,5-compound with zinc chloride in acetone gave the 3,4-isomer, while longer reaction time gave the 2,3:4,5 product which was also formed on similar treatment of the 3,4-monosubstituted acetal. Conversely, 2,4:3,5-di-*O*-benzylidene-D-xylose dialkyl acetals were the products of treatment of the free sugar with benzaldehyde–primary alcohol mixtures.[206] Acetone also condensed with xylitol and D-arabinitol in the presence of sulphuric acid and copper sulphate to give 2,3:4,5-derivatives which were characterised by benzylation followed by hydrolysis to the 1-*O*-benzyl ethers. 2-*O*-Benzyl-D-arabinitol and 3-*O*-benzylxylitol were both described in this report.[209] The acid-catalysed reaction of chloroacetaldehyde diethyl acetal with D-galactitol gave the 1,3:4,6-acetal, the di-*O*-toluene-*p*-sulphonyl derivative of which gave the known 1,3,4,6-tetra-*O*-acetyl-2,5-di-*O*-toluene-*p*-sulphonyl-D-galactitol on acetolysis.[210]

[206] R. J. Ferrier and L. R. Hatton, *Carbohydrate Res.*, 1967, **5**, 132.
[207] D. G. Lance and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 1533.
[208] H. Zinner and J. Milbradt, *Carbohydrate Res.*, 1967, **3**, 389.
[209] T. Nakagawa, H. Tokuoka, K. Shinoto, J. Yoshimura, and T. Sato, *Bull. Chem. Soc. Japan*, 1967, **40**, 2150.
[210] H. B. Sinclair and W. J. Wheadon, *Carbohydrate Res.*, 1967, **4**, 292.

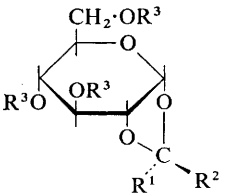
Bourne and his co-workers [211] have reported on the action of mixtures of trifluoroacetic anhydride and acetic acid on 1,3:2,5:4,6-tri-*O*-methylene-D-mannitol. Partial deacetalation occurred and three functional groups of interest were found in the products, namely, acetoxymethyl, alkoxymethyl, and oxydimethylene, as well as acetyl groups. A reaction scheme was proposed in which the first products of ring cleavage were *O*-acetyl and *O*-trifluoroacetoxymethyl derivatives, and the latter then broke down as shown to give acetoxymethyl or alkoxymethyl compounds, the alcohol being available in the isolation procedures. Where there were two sterically suitably situated groups the oxydimethylene ring system (59) was formed.[211]



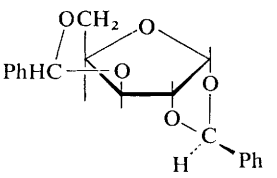


(59)

From Diols on Cyclic Carbohydrates.—(a) *Free sugars.* The homomorphology of sugar acetals has been reviewed (in Czech).[212] A new method for obtaining cyclic acetals attached to the anomeric centre has been developed by treating tetra-*O*-acyl-α- (and β-) -D-glucopyranosyl bromides with cadmium dialkyls. Thus, for example, the acetyl derivative with diethyl cadmium gave (60) whilst the propionyl compound with dimethylcadmium gave (61). De-esterification of (60) and (61) gave diastereoisomers. The configurations were established by n.m.r. methods which further suggested that the pyranose rings of the products existed in a flattened chair conformation.[213]



(60) R^1 = Me, R^2 = Et, R^3 = Ac
(61) R^1 = Et, R^2 = Me, R^3 = COEt



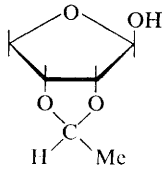
(62)

[211] T. G. Bonner, E. J. Bourne, and D. Lewis, *J. Chem. Soc.* (*C*), 1967, 2321.
[212] J. Pacák, M. Černý, and J. Staněk, *Chem. listy*, 1967, **61**, 191.
[213] R. G. Rees, A. R. Tatchell, and R. D. Wells, *J. Chem. Soc.* (*C*), 1967, 1768.

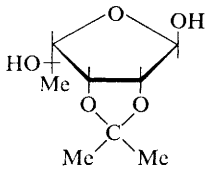
By more conventional means involving the acid-catalysed benzylidenation of D-xylose, two diastereoisomeric 1,2:3,5-substituted derivatives were produced, and were shown to differ only in the stereochemistry at the 1,2-acetal centre, the major product having the phenyl group *exo* (62).[206] N.m.r. also provided means for assigning conformation to the six-membered rings.

The reaction of acetone dimethyl acetal with L-sorbose in the presence of toluene-*p*-sulphonic acid has been shown to yield different mixtures of products at different temperatures.[214] At room temperature the products were the 1,2:3,4- and 1,2:4,5-di-*O*-isopropylidene-α-pyranose derivatives, together with the 5-*O*-(1′-methoxyisopropyl)-1,2:3,4-di-*O*-isopropylidene-α-pyranose compound, whereas at 45°, 1,3:4,6-β-, 1,2:4,6-α- and -β-, and 2,3:4,6-α-furanose compounds were obtained. At reflux temperature the 2,3:4,6-α-furanose acetal together with the methyl glycoside of 1,3:4,6-di-*O*-isopropylidene-β-L-sorbofuranose were produced. 1,2:4,5-Di-*O*-cyclohexylidene-D-fructose has been shown to give the crystalline 1,2-acetal, isolated in 38% yield.[215] 1,2:4,6-Di-*O*-isopropylidene-L-sorbose has been characterised by mass spectrometry.[216]

Compounds with acetal rings at positions other than the anomeric centre of cyclic sugars have been investigated. 2,3-*O*-Ethylidene-D-erythrose (63) was shown to be the product both from reaction of the free sugar with paraldehyde in aqueous acid and from the acid-catalysed rearrangement of the 2,4-*O*-acetal. N.m.r. spectroscopy showed that compound (63) and its derivatives have the β-configuration as would be expected on steric



(63)



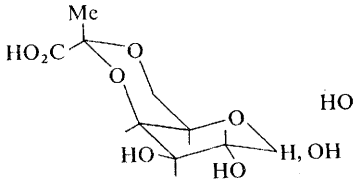
(64)

grounds; the crystalline free sugar did not mutarotate.[217] This was consistent with the finding that very little β-furanose was present in the equilibrium established by 2,3-*O*-isopropylidene-L-rhamnose in aqueous solution.[218] The α-furanose (64) was the main constituent (65%), together with the α- and β-pyranoses (25% and 10%, respectively). A little 2-hydroxyquinoline added to the ether–light petroleum used for crystallising compound (64) catalysed the mutarotation and greatly facilitated the crystallisation process. This technique was recommended for sugars having

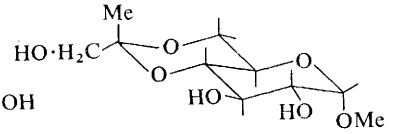
[214] T. Maeda, *Bull. Chem. Soc. Japan*, 1967, **40**, 2122.
[215] N. P. Klyushnik, *Ukrain. khim. Zhur.*, 1967, **33**, 67.
[216] J. R. Patil, K. G. Das, and J. L. Bose, *Indian J. Chem.*, 1967, **5**, 535.
[217] J. W. Van Cleve and C. E. Rist, *Carbohydrate Res.*, 1967, **4**, 82.
[218] S. J. Angyal, V. A. Pickles, and R. Ahluwahlia, *Carbohydrate Res.*, 1967, **3**, 300.

a free reducing function which are crystallised from non-hydroxylic solvents.

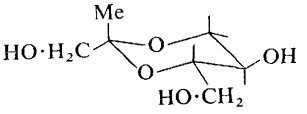
4,6-*O*-(1-Carboxyethylidene)-D-galactose (65) obtained from a bacterial polysaccharide hydrolysate has been configurationally characterised at the acetal centre. Compound (66), obtained synthetically, gave, on periodate oxidation and reduction, the diol (67) which was also obtained



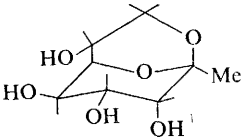
(65)



(66)



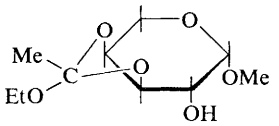
(67)



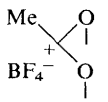
(68)

from 2,8-anhydro-1-deoxy-D-*glycero*-β-D-*gulo*-octulopyranose (68). Consequently (66) was configurationally characterised. Isomers in the D-galactose series prepared from (65) were then related to derivatives of (66) by rotational comparisons and by intramolecular hydrogen bonding studies.[219] In general, axial *C*-methyl groups on six-membered rings were found to give n.m.r. signals at lower fields than equatorial groups.[219]

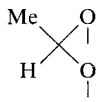
(b) *Glycosides.* A method of preparing acetals from orthoesters has been applied for the first time to carbohydrate systems. Methyl 3,4-*O*-ethoxyethylidene-β-L-arabinopyranoside, prepared by orthoester exchange using triethylorthoacetate in the presence of toluene-*p*-sulphonic acid, was shown



(69)



(70)



(71)

to be a mixture of *endo*-methyl and *exo*-methyl isomers (69) in the ratio 5 : 1, which on treatment with boron trifluoride etherate gave a gummy fluoroborate salt (70) which on reduction with lithium borohydride in ether gave the ethylidene acetal (71) in crystalline form, together with

[219] P. A. J. Gorin and T. Ishikawa, *Canad. J. Chem.*, 1967, **45**, 521.

minor amounts of the diastereoisomer (ratio 9 : 1).[220] Methyl 4-*O*-acetyl-2,3-anhydro-β-L-lyxopyranoside on treatment with boron trifluoride also gave a gummy precipitate which when reduced with lithium borohydride gave the same crystalline acetal, thereby proving participation of the acetoxy-group in the original epoxide ring-opening. N.m.r. spectroscopy afforded a means for assigning configurations at the acetal and orthoester asymmetric centres.[220]

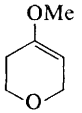
4,6-Acetals of methyl α-D-glucopyranoside have been prepared by acid-catalysed acetal exchange with the dimethyl acetals of acetone, pinacolone, acetophenone, and benzophenone. In the case of the first two ketones, 2,3:4,6-diacetals were isolated in minor amounts. Benzophenone dimethyl acetal gave methyl 6-*O*-(methoxydiphenylmethyl)-α-D-glucopyranoside as a minor product. The 4,6-acetals were hydrolysed as their 2,3-di-*O*-methyl ethers by 75% acetic acid, and whereas the alkyl ketone derivatives reacted in 90 min., those derived from acetophenone and benzophenone took several days.[221] The same group of workers has shown that reaction of polyols with 1,1-dimethoxycyclohexane and toluene-*p*-sulphonic acid in DMF, in a system in which the methanol was removed, gave cyclic acetals in high yields even when these bridged vicinal *trans*-diols on six-membered rings. Methyl α-D-glucopyranoside gave the 2,3:4,6-diacetal in 93% yield, and methyl α-D-xylopyranoside gave, after toluene-*p*-sulphonylation of the products, a mixture of the 2,3-*O*-cyclohexylidene 4-toluene-*p*-sulphonate and the 3,4-*O*-cyclohexylidene 2-toluene-*p*-sulphonate.[222] Acetals of *trans*-diols on six-membered rings have also been synthesised under basic conditions: reaction of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and the corresponding D-galactoside derivative with methylene halides and sodium hydride in DMF gave the corresponding 2,3-*O*-methylene compounds.[223]

Methyl α-D-glucopyranoside (and D-glucose and D-glucitol) have been reacted with long-chain aldehydes (and their methyl acetals) to give acetal derivatives of potential commercial value.[224]

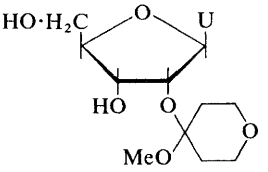
The solvolyses of 4,6-*O*-benzylidene-hexosides have been investigated using polarography to monitor the liberated benzaldehyde, and it was concluded that (i) *cis*-fused benzylidine rings were more stable than *trans*-fused rings, (ii) only slight effects were produced on altering configuration at C-1, C-2 and C-3, (iii) hydroxy-compounds reacted faster than acetylated or benzoylated systems, and (iv) substitution of hydroxy-groups at C-2 and C-3 by amino-groups caused a decrease in the reaction rates. The mechanism of hydrolysis was discussed in terms of the basicity of the acetal oxygen atoms.[225]

[220] J. G. Buchanan and A. R. Edgar, *Chem. Comm.*, 1967, 29.
[221] M. E. Evans, F. W. Parrish, and L. Long, jun., *Carbohydrate Res.*, 1967, **4**, 453.
[222] F. H. Bissett, M. E. Evans, and F. W. Parrish, *Carbohydrate Res.*, 1967, **5**, 184.
[223] J. S. Brimacombe, A. B. Foster, B. D. Jones, and J. J. Willard, *J. Chem. Soc.* (*C*), 1967, 2404.
[224] R. E. Sharpe, D. A. Berry, E. H. Pryde, and J. C. Cowan, *J. Amer. Oil Chemists' Soc.*, 1967, **44**, 167.
[225] J. Kovář and J. Jarý, *Coll. Czech. Chem. Comm.*, 1967, **32**, 854.

From Single Alcoholic Groups.—Tetrahydropyranyl acetals may be used to protect individual hydroxy-groups during synthetic work. However, with optically active alcohols, dihydropyran has the disadvantageous property of yielding mixtures of diastereoisomeric acetals. The problem may be overcome by the use of symmetrical acetals and for this purpose the 2′-acetone and 2′-cyclohexanone methylacetals of uridine were prepared by way of 3′,5′-di-*O*-acetyluridine. These were, however, found to be too sensitive to acid hydrolysis to serve as suitable protecting groups in oligonucleotide synthesis, and so the vinyl ether (72) was selected for the pre-



(72)



(73)

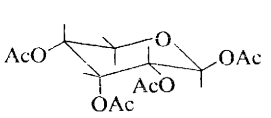
paration of methoxytetrahydropyranyl derivatives such as (73). This symmetrical protecting group possessed nearly optimal stability for use in oligonucleotide syntheses,[226] and should find further use in carbohydrate research.

[226] C. B. Reese, R. Saffhill, and J. E. Sulston, *J. Amer. Chem. Soc.*, 1967, **89**, 3366.

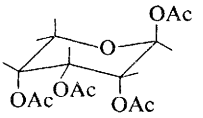
6
Esters

Acetates

A review of acetolysis reactions has appeared.[227] Two papers of outstanding interest are dealt with in this Section. For the first time direct proof has been obtained that pyranoid sugars at normal temperatures exist in both chair conformations in equilibrium with each other, and that n.m.r. spectra measured under usual conditions represent the time average of the spectra of both modifications.[228] The 220 Mc./sec. spectrum of tetra-*O*-acetyl-β-D-ribopyranose was completely first order at room temperature, and the spin–spin couplings were intermediate between those expected for



(74)

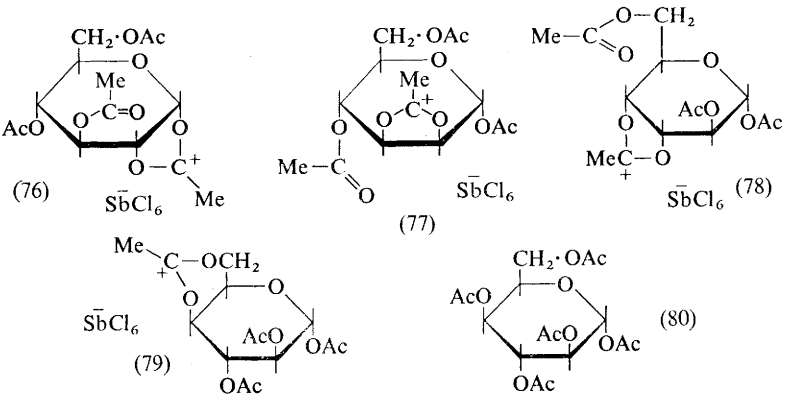


(75)

the *C*1 and 1*C* conformations (74) and (75). As the temperature of measurement was lowered, however, the spectrum resolved into the sum of the spectra of each chair form. The anomeric proton, for example, which showed as a doublet ($J_{1,2}$ 4·8 c./sec.) at room temperature, appeared as two doublets ($J_{1,2}$ *ca.* 1 c./sec. and 8·0 c./sec. respectively) at −84°, assigned to the 1*C* (75) and *C*1 (74) chairs, and of relative intensities 2 : 1. The remainder of the spectrum resolved similarly. It was concluded that the 1*C* conformation was favoured by some 0·3 Kcal./mole at this temperature, and so this work would appear to open the way to a further understanding of the quantitative aspects of carbohydrate stereochemistry. The spectrum of tetra-*O*-acetyl-α-D-lyxopyranose did not resolve at low temperatures indicating that if any of the 1*C* form was present it cannot comprise more than 2% of the total. Both the *ribo*- and *lyxo*-peracetates therefore favour the chair conformation with the oxygenated group at C-1 axial. In other work chemical shifts of acetate protons have been considered in detail (see p. 182).

[227] R. D. Guthrie and J. F. McCarthy, *Adv. Carbohydrate Chem.*, 1967, **22**, 11.
[228] N. S. Bhacca and D. Horton, *J. Amer. Chem. Soc.*, 1967, **89**, 5993.

An extensive series of isomerisations occurred on treating penta-*O*-acetyl-β-D-glucopyranose or tetra-*O*-acetyl-α- or β-D-glucopyranosyl chloride in methylene chloride solution with antimony pentachloride. Initially the acetoxonium salt (76) was formed and this isomerised *via* the *manno*- (77) and *altro*- (78) isomers to the D-*ido*-compound (79) which



crystallised from solution in *ca.* 65% yield.[229] The n.m.r. spectrum agreed with the allocated structure, conclusive proof being obtained by examining the spectrum of the salt derived after C-6-acetyl deuteriation of the D-glucose starting material. In this case, the methyl resonance of the acetoxonium system was missing. Hydrolysis of the salt (79) followed by acetylation gave penta-*O*-acetyl-D-idopyranose (80) in good yield, ethanolysis gave an idose 4,6-orthoester derivative, but treatment with acetic anhydride caused a reversal of the isomerisation sequence and gave penta-*O*-acetyl-α-D-glucopyranose. The salt isolated on treatment of tetra-*O*-acetyl-β-D-glucopyranosyl fluoride with boron trifluoride etherate had not isomerised and was shown to have a 1,2-acetoxonium ionic structure. 1,2,3,6-Tetra-*O*-acetyl-α-D-idopyranose was shown by n.m.r. studies to adopt the *C*1 chair form in solution.

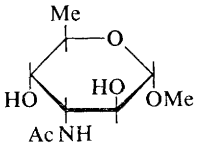
Another type of acetate isomerisation reaction has been reported to occur when β-lactose octa-acetate was treated with various clays in chloroform solution. 4-*O*-(β-D-Galactosyl)-D-mannose octa-acetate was isolated in 61% yield which implied that a contrathermodynamic process was operating.[230]

Studies on the partial deacetylation of carbohydrate esters have been reported. It was shown[231] that the octa-acetates of β-cellobiose and β-maltose on treatment with piperidine in THF each gave a hepta-acetate,

[229] H. Paulsen, W.-P. Trantwein, F. G. Espinosa, and K. Heyns, *Chem. Ber.*, 1967, **100**, 2822.
[230] N. V. Aleksidze, *Zhur. obshchei Khim.*, 1967, **37**, 2625.
[231] R. M. Rowell and M. S. Feather, *Carbohydrate Res.*, 1967, **4**, 486.

which were shown by n.m.r. and chemical methods to be unsubstituted at the reducing centre and to have the α- and β-configuration respectively. Methylation of either product yielded a mixture of approximately equal proportions of α- and β-glycosides. Similar reactions occurred with β-melibiose and β-lactose octa-acetates, but the products from these esters were not completely characterised. It has also been reported briefly [232] that anion exchange resins can be used in deacetylations, and from penta-*O*-acetyl-β-D-galactopyranose the free sugar and its 6-acetate were obtained in addition to other partially deacetylated compounds. It was recommended that before location of highly substituted compounds on chromatograms was attempted these should be sprayed with sodium hydroxide (0·5%) in ethanol and dried at 100°.[232]

Partial acetylations have been described. 3-(β-D-Xylofuranosyl)uracil, for example, gave predominantly the 2′-acetate.[233] In this work the 2′- and 3′-monoacetates of this nucleoside were prepared *via* the 3′,5′-*O*-isopropylidene and 2′,5′-di-*O*-trityl compounds, respectively. When either acetate was heated in pyridine under reflux the 5′-acetate was obtained which illustrated the known propensity for ester functions to migrate to a primary hydroxy-group. Partial acetylation of methyl 3-acetamido-3,6-dideoxy-α-D-altropyranoside (81) showed, as has been observed previously,



(81)

that the course of the reaction was dependent upon the reagents used; acetic anhydride and pyridine yielded 36% of the diacetate, together with 54% of the 4-acetate, a little unacetylated material, and none of the 2-ester. Alternatively, acetyl chloride and pyridine reacted at positions 2 and 4 at comparable speeds, and the products comprised diacetate (25%), 4-acetate (25%), and 2-acetate (32%).[234]

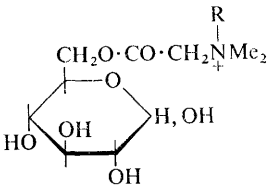
Complete acetylation of aldoses and 2-amino-2-deoxyaldoses with acetic anhydride in pyridine was shown to occur mainly without mutarotation.[235] The acetyl resonances of the products are discussed on p. 182.

Substituted Acetates and Other Nonaromatic Carboxylates

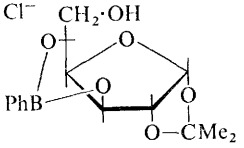
Trifluoroacetylation of thymidine with phenyl trifluoroacetate in pyridine caused disubstitution even when molar proportions of acylating agent were used; other per(trifluoroacetates) of nucleosides have been described.[236]

[232] Y. Z. Frohwein, *Israel J. Chem.*, 1967, **5**, 141P.
[233] G. A. R. Johnston, *Tetrahedron Letters*, 1967, 2679.
[234] K. Čapek, J. Šteffková, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1967, **32**, 249.
[235] D. Horton, J. B. Hughes, J. S. Jewell, K. D. Philips, and W. N. Turner, *J. Org. Chem.*, 1967, **32**, 1073.
[236] G. Kresze, E. Lodemann, and A. Wacker, *Z. Naturforsch.*, 1967, *B*, **22**, 285.

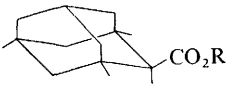
Chloroacetylation of carbohydrates can be achieved using chloroacetic anhydride in pyridine solution. The reagent has now been used to prepare the 6-chloroacetate of 1,2-*O*-isopropylidene-α-D-glucofuranose in which positions 3 and 5 were protected with boric acid. In this case, chloroacetylation with the free acid and DCC failed.[237] The 6-chloroacetate of D-glucose has been prepared by the same authors and was converted to the quaternary ammonium salt (82), whose biological activity was compared with that of the hydrochloride of 6-*O*-(*NN*-dimethylglycyl)-α-D-glucose



(82) R = Me
(83) R = H



(84)



(85)

(83), prepared from D-glucose 1,2:3,5-bisphenylboronate (84) and the appropriate acid by a procedure using DCC.[238] The synthesis of 6-*O*-(*NN*-dialkylglycyl) esters of D-glucono-γ-lactone and D-gluconamide has been described.[239]

Adamantoyl esters (85) have been shown to be useful derivatives that may be considered to be the base-labile counterparts of trityl ethers. Treatment of 2′-deoxyribonucleosides with adamantoyl chloride in pyridine solution caused preferential substitution at the primary (5′) position and provided suitable means for obtaining mono-*O*-substituted derivatives.[240] Alternatively, when the 5′-site was tritylated the reagent caused substitution at the 3′-position. Trityl ethers and acetyl esters could be removed (with mild acid and alkali, respectively) in the presence of the adamantoate group which could then be cleaved with stronger base. Its use was demonstrated in the synthesis of thymidine 3′-phosphate, and 5′-adamantoates of ribonucleosides were prepared from 2,3-*O*-isopropylidene derivatives.[240]

Long-chain fatty acid esters have been investigated. D-Arabinofuranoses esterified at the primary hydroxy-group by aliphatic acids of chain length *ca.* 90 have been isolated from bacterial lipids and examined over the range *m/e* 1050 to 1450 by mass spectrometry. Peaks derived from two esters were recognised and the sugar was assigned the furanose ring form.[241] Related compounds are of commercial significance and a new

[237] S. G. Verenikina, A. M. Yurkevich, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1967, **37**, 1458.
[238] S. G. Verenikina, A. M. Yurkevich, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1967, **37**, 2181.
[239] A. M. Yurkevich, S. G. Verenikina, M. S. Dolgich, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1967, **37**, 1267.
[240] K. Gerzon and D. Kau, *J. Medicin. Chem.*, 1967, **10**, 189.
[241] N. P. V. Acharya, M. Senn, and E. Lederer, *Compt. rend.*, 1967, *C*, **264**, 2173.

process has been described for the preparation of fatty-acid esters of sucrose. Based on emulsifying techniques, it can be used to prepare sucrose monostearate in 15% yield.[242] Other long-chain fatty-acid esters of sucrose were described[243] in which 50% substitution occurred at C-6 of glucose, 10% at other glucose positions, 40% at C-6 of fructose, and 5% at other fructose sites.

Benzoates

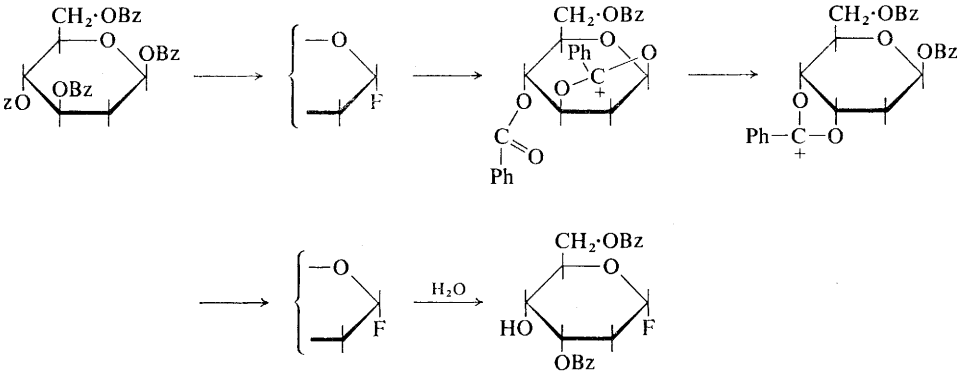
A novel approach to the investigation of selective esterification has been adopted by examining the products of multiple substitution rather than those of monosubstitution. Esterification of methyl α-D-glucopyranoside, -galactopyranoside, or -mannopyranoside with four molar equivalents of benzoyl chloride in pyridine gave 2,3,6-triesters in good yield (>50% in each case). In addition, the 2,4,6-isomer was isolated in 19% yield from reaction with the glucoside. Dibenzoylation of the glucoside and mannoside gave mainly 2,6- and 3,6-diesters respectively, but the galactoside showed poor selectivity.[244] From these results it was deduced that the orders of reactivity of the hydroxy-groups were: glucoside, 2>3>4; galactoside, 2,3>4; mannoside, 3>2>4. The reasons for these sequences were discussed, and it was noted that it is not a generalisation to consider the hydroxy-group on C-2 as the most reactive group. Steric factors were taken as being most important, and the likelihood of benzoyl migration occurring was taken to be small. N.m.r. was shown to be of the greatest value in the characterisation of the partially substituted products.

Extension of these studies to methyl 6-deoxy-α-L-galactopyranoside and -mannopyranoside showed that dibenzoylation gave 2,3-esters in good yield in both cases (80 and 50% respectively).[245] From the former the tribenzoate and 3-mono-*O*-benzoate were also obtained in 11 and 6% yields. It was concluded, by comparison of the hexoside and deoxyhexoside results, that CH_2OBz and CH_3 groups at C-5 behaved similarly as far as their influence on substitution at C-2, C-3, and C-4 was concerned. Preferential benzoylation of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside gave a 2-ester in keeping with these findings.[246] Other workers have applied these methods to benzyl β-L-arabinopyranoside and benzyl α-D-xylopyranoside[247] and have shown that dibenzoylation of the former gave the crystalline 2,3-ester in 65–67% yield, together with the fully substituted arabinoside (10–15%). The main product therefore arose by preferential reaction at the equatorial hydroxy-groups and the result conformed with expectations based on the galactoside result,[244] and with work carried out on methyl β-L-arabinopyranoside which afforded a

[242] L. I. Osipow and W. Rosenblatt, *J. Amer. Oil Chemists' Soc.*, 1967, **44**, 307.
[243] E. Tamate and T. Otake, *Yukagaku*, 1967, **16**, 395.
[244] J. M. Williams and A. C. Richardson, *Tetrahedron*, 1967, **23**, 1369.
[245] A. C. Richardson and J. M. Williams, *Tetrahedron*, 1967, **23**, 1641.
[246] A. C. Richardson, *Carbohydrate Res.*, 1967, **4**, 415.
[247] T. Sivakumaran and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 2493.

means for introduction of an amino-function specifically at C-4.[248] Similar treatment[247] of benzyl α-D-xylopyranoside gave the triester (15%), a syrupy diester shown to be the 2,4-derivative (45%), a crystalline isomer (27%, 2,3-diester), and benzyl 2-*O*-benzoyl-α-D-xylopyranoside (9%). The major product formed is in contrast with the 2,3,6-tri-*O*-benzoyl derivative formed preferentially from methyl α-D-glucopyranoside[244] and a steric explanation was offered for this apparent discrepancy. The partially substituted compounds were characterised in the main by forming from them, methanesulphonates, which were then subjected to nucleophilic displacement reactions to give compounds, whose configurations showed the location of the sulphonate groups.[247]

Improved methods for the synthesis of the anomeric tetra-*O*-benzoyl-D-xylofuranoses from methyl α,β-D-xylofuranoside have been reported.[249] Tetra-*O*-benzoyl-2-deoxy-β-D-*arabino*-hexopyranose gave the corresponding α-glycosyl fluoride on treatment with hydrogen fluoride in benzene, but in anhydrous hydrogen fluoride the benzoate was converted to 3,6-di-*O*-benzoyl-2-deoxy-α-D-*ribo*-hexopyranosyl fluoride isolated in 62% yield.[250] The reaction and the corresponding process undergone by the analogous acetate were followed by n.m.r. spectroscopy and the mechanism shown in Scheme 10 was proposed for the benzoate conversion. An example of the



Scheme 10

use of the thionobenzoate group for the introduction of a thiol group on to a carbohydrate ring is given on p. 72.

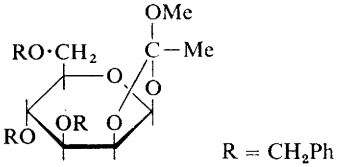
In a continuation of their studies on the intramolecular migration of benzoyl groups during ammonolysis of esterified sugars Deulofeu and his group have shown, using labelling techniques, that a partial benzoyl migration from C-4 to C-6 occurred during the conversion of penta-*O*-benzoyl-D-glucopyranose to 1,1-bis(benzamido)-1-deoxy-D-glucitol.

[248] E. J. Reist, L. V. Fisher, and L. Goodman, *J. Org. Chem.*, 1967, **32**, 2541.
[249] H. G. Fletcher, jun., and H. W. Diehl, *Carbohydrate Res.*, 1967, **4**, 438.
[250] I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 1967, **21**, 1239.

Migration from C-2 to C-6 was detected in similar fashion during the ammonolysis of penta-*O*-benzoyl-*aldehydo*-D-glucose.[251] (See also p. 111.) A benzoate migration also occurred during mild acid hydrolysis of 2,4-*O*-ethylidene-1,3-di-*O*-*p*-nitrobenzoyl-D-erythritol, and instead of the expected 1,3-diester, 1,4-di-*O*-*p*-nitrobenzoyl-D-erythritol was obtained.[252]

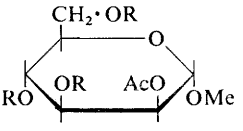
Carboxylic Orthoesters

A synthesis of cyclic orthoesters by a transesterification procedure using triethyl orthoacetate and their reaction *via* acyloxonium ions to give cyclic acetals has also been mentioned.[220] (See also ref. 229.) The importance of compounds in this series in glycoside and disaccharide synthesis has already been discussed (see p. 18), and it has also been shown that simple treatment with acid converts glycosyl 1,2-orthoesters into glycosides. Thus, the ring-opening of 3,4,6-tri-*O*-benzyl-β-D-mannopyranose 1,2-(methyl orthoacetate) (86) in dichloromethane with toluene-*p*-sulphonic acid as



(86)

R = CH_2Ph



(87)

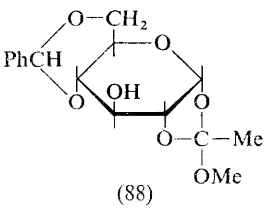
catalyst and in the absence of water or alcohols gave predominantly methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannoside (87) with the deacetylated analogue as a minor product.[253] The only effect of the addition of methanol to the reaction system was to increase the amount of deacetylated compound. Experiments carried out on the cyclohexyl and isopropyl orthoesters gave similar results. β-Gentiobiose octa-acetate has been synthesised by this method.[97]

An unusual type of trimeric compound has been reported from the reaction of the bifunctional derivative (88) under transesterifying conditions. Saponification of the acetate followed by treatment with mercuric bromide in nitromethane gave the 15-membered ring compound (89), the structure of which was established by hydrolysis, n.m.r. spectroscopy, and molecular weight determination.[254]

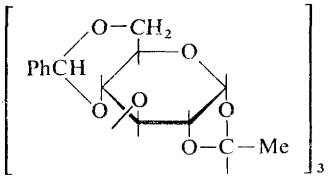
Orthoesters have been evaluated as blocking groups for the *cis*-2′,3′-diol of ribonucleosides in oligonucleotide syntheses. Orthoester exchange of nucleosides with trimethyl orthoformate gave 2′,3′-*O*-methoxymethylidene derivatives in good yields.[255] Dilute acids caused hydrolysis to mixtures of

[251] A. Lezerovich, E. G. Gros, J. F. Sproviero, and V. Deulofeu, *Carbohydrate Res.*, 1967, **4**, 1.
[252] J. W. Van Cleve and C. E. Rist, *Carbohydrate Res.*, 1967, **4**, 95.
[253] N. E. Franks and R. Montgomery, *Carbohydrate Res.*, 1967, **3**, 511.
[254] N. K. Kochetkov and A. F. Bochkov, *Tetrahedron Letters*, 1967, 4669.
[255] B. E. Griffin, M. Jarman, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2301.

2′- and 3′-formyl esters under very mild conditions and these then hydrolysed readily to free nucleosides at pH$\geqslant$7. It was shown that in the presence of the cyclic protecting groups, substitution, in particular phosphorylation, could be brought about at the 5′-position prior to specific removal of the orthoester. During this work[255] a pure diastereoisomer



(88)



(89)

(with defined configuration at the new asymmetric centre) was isolated, but unresolved mixtures were satisfactory for synthetic purposes.

Carbonates

The first naturally occurring sugar carbonate has been found in an antibiotic (see p. 163).

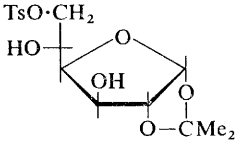
In a paper which summarised the various protecting groups used in nucleoside synthetic work, the value of *p*-nitrophenyl chloroformate was assessed as a blocking reagent. With compounds containing isolated hydroxy-groups, *p*-nitrophenyl carbonates were formed and were similar to the previously described dinitrobenzenesulphinic esters in their value. The reagent, used in pyridine solution, gave carbonate esters that were stable to mild acid and to phosphorylating conditions, and which could be removed by treatment with imidazole, which did not cleave acetates or benzoates.[256] Thymidine 5′-phosphate was prepared in good yield starting from 5′-*O*-tritylthymidine by protection of the 3′-position with the carbonate ester, removal of the ether group, phosphorylation, and final decarbonation. Alternatively, the 3′-phosphate was obtained by preferential 5′-esterification of thymidine with *p*-nitrophenyl chloroformate (33% yield) followed by 3′-phosphorylation and alkaline cleavage of the 5′-group. With ribonucleosides the reagent gave 2′,3′-cyclic carbonates in good yields,[256] and these were valuable base-labile derivatives. The chloroformate was particularly useful in cyclic carbonate formation with those compounds which readily formed 2,2′-anhydrides, *e.g.* uridine.

Isobutyl chloroformate reacted similarly with thymidine in pyridine to give isobutyl thymidine 5′-carbonate in 73% yield, but when the 5′-position was protected by initial tritylation, substitution at the 3′-position occurred as before, and acid-catalysed removal of the ether yielded the 3′-carbonate.

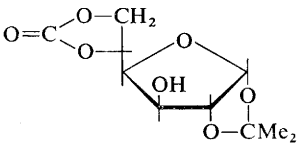
[256] R. L. Letsinger and K. K. Ogilvie, *J. Org. Chem.*, 1967, **32**, 296.

The isobutyl carbonate group was also shown to be suitable for protection of hydroxy-groups during phosphorylation.[257]

Cyclic carbonates can be synthesised very satisfactorily from primary sulphonyl esters which have neighbouring hydroxy-groups. Potassium hydrogen carbonate in DMSO caused the reaction to proceed at room temperature. 1,2-*O*-Isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose (90), for example, was converted in 85% yield to the 5,6-carbonate (91). At higher temperatures, however, both compounds (90) and (91)



(90)



(91)

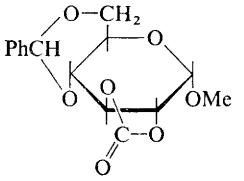
gave the 3,6-anhydro-derivative by nucleophilic attack of the hydroxy-group at C-3 on C-6. Similarly, the 6-sulphonyl ester of D-glucose phenylosotriazole gave first the 5,6-cyclic carbonate and then the 3,6-anhydride, and the 1,6-disulphonate of D-mannitol gave the 1,2:5,6- dicarbonate from which 3,4-*O*-isopropylidene-D-mannitol was obtained.[258]

The reaction of excess of ethyl chloroformate with pyranoid compounds containing vicinal diequatorial hydroxy-groups gave *trans*-five-membered cyclic derivatives in the presence of triethylamine. Methyl 4,6-*O*-benzylidene-α-D-glucoside gave the 2,3-carbonate (92) and methyl 2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside gave the 3,4-cyclic ester.[259] In the presence of pyridine, on the other hand, acyclic derivatives were formed. Thus, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside gave the 2,3-di-*O*-ethoxycarbonyl derivative or, with limiting amounts of the reagent, the 2- and 3-monoesters in the proportions 24 : 1.[259] These were separated by column chromatography and were found not to give the cyclic compound on treatment with triethylamine. Reaction of methyl 6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside with ethyl chloroformate in triethylamine gave a product assumed to be methyl 4-*O*-ethoxycarbonyl-6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside 2,3-carbonate. The i.r. spectra of *cis*- and *trans*-fused cyclic carbonates were compared and it was suggested that a band in the region 1840–1844 cm.$^{-1}$ was characteristic of the latter group.[259]

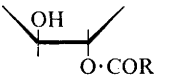
The cyclic carbonate (92) underwent ring-opening on treatment with, for example, methanol, toluenethiol, or piperidine to give mixtures of 2- and 3-substituted derivatives (93) and (94), but the corresponding *manno*-isomer of (92), with a *cis*-fused carbonate ring, underwent ring-opening

[257] K. K. Ogilvie and R. L. Letsinger, *J. Org. Chem.*, 1967, **32**, 2365.
[258] G. Hanisch and G. Henseke, *Chem. Ber.*, 1967, **100**, 3225.
[259] W. M. Doane, B. S. Shasha, E. I. Stout, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **4**, 445.

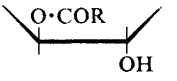
much less readily.[260] In an extension of this work it was shown that carbohydrates could be condensed with starch through carbonate bridges; compound (92) [and its thio-analogue (95)] reacted with starch in aqueous DMSO in the presence of triethylamine as catalyst.[261]



(92)

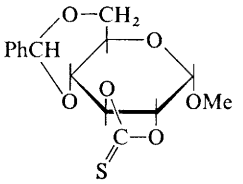


(93)

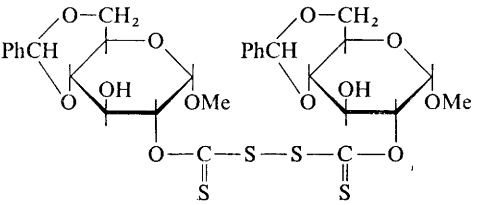


(94)

Thermal or catalysed decomposition of 6-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose gave the bis-6,6′-dicarbonate, and not the hoped-for 6-deoxy-6-fluoro-derivative; a similar reaction was observed with 5-*O*-fluoroformyl-1,2:3,4-di-*O*-isopropylidene-DL-xylitol.[262]



(95)



(96)

Thiocarbonates

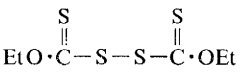
Reports on a variety of sulphur analogues of carbonates have appeared. Treatment of the dimeric compound (96) with pyridine gave the cyclic thionocarbonate (95) in high yield, which with silver nitrate yielded the carbonate analogue (92), again in excellent yield, providing a new route to these compounds.[263] By its application 2,3-thionocarbonate and 2,3-carbonate groups have been introduced into 6-*O*-trityl-amylose.

Attempts to prepare the 5,6-thionocarbonate of 1,2-*O*-isopropylidene-α-D-glucofuranose by treating the 3,5,6-trihydroxy-compound with *OO*′-diethyl dithiobis(thioformate) (97) in the presence of pyridine resulted in the isolation of the 5,6-carbonate (30%), which was produced in improved yield by the use of triethylamine in acetone or in DMSO, and so a further

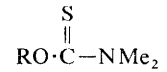
[260] E. I. Stout, W. M. Doane, B. S. Shasha, C. R. Russell, and C. E. Rist, *Tetrahedron Letters*, 1967, 4481.
[261] W. M. Doane, E. I. Stout, B. S. Shasha, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **5**, 366.
[262] N. Baggett, K. W. Buck, A. B. Foster, R. Jefferis, and J. M. Webber, *Carbohydrate Res.*, 1967, **4**, 343.
[263] E. I. Stout, W. M. Doane, B. S. Shasha, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **3**, 354.

general synthesis of cyclic carbonates was suggested. The thionocarbonate was not an intermediate in the carbonate formation.[264]

A number of substituted monosaccharides, each having one free hydroxy-group, have been converted into the corresponding crystalline dimethyl-thiocarbamates (98) (which can be considered to be nitrogen analogues of



(97)



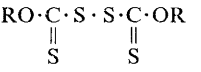
(98)

thionocarbonates), by conversion into the *O*-sodio-derivatives followed by treatment with dimethylthiocarbonyl chloride.[265] N.m.r. studies showed that the two *N*-methyl groups were nonequivalent at 45° but become indistinguishable above 110°; the spectra of the products were discussed in some detail.

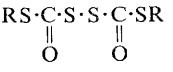
The related alkylthiocarbonyl esters can be converted into cyclic carbonates as in the case of methyl 3-*O*-benzylthiocarbonyl-4,6-*O*-benzylidene-β-D-galactoside which gave the 2,3-carbonate on pyrolysis.[266]

Xanthates, perhaps the most important esters in this series, have received further attention. As a model for the reaction of starch, the xanthation of methyl α-D-glucopyranoside has been studied using glucoside, sodium hydroxide, and carbon disulphide in the ratios 2 : 2 : 1. Throughout the reaction the 6-isomer predominated and esterification occurred more-readily at C-2 than at C-3. During the initial stages, the sum of the secondary substitution rates and the reaction rate at the primary position were about the same, but after longer times the primary rate apparently exceeded the secondary one. This was believed to be due to migration of ester groups since experiments with authentic 2-, 3-, and 6- monoesters showed that migration occurred to the primary position, most probably by the route 2 → 3 → 6.[267]

The same group of workers prepared the xanthate from 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose and oxidised it with iodine to find none



(99)

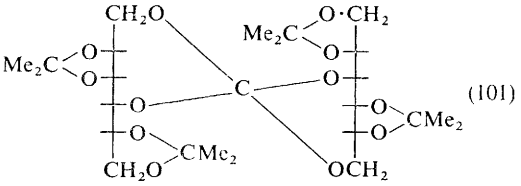


(100)

of the expected bis-(*O*-thiocarbonyl) disulphide derivative (99) but rather the rearranged product (100), which, on reductive desulphurisation and

[264] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **5**, 346.
[265] D. Horton and H. S. Prihar, *Carbohydrate Res.*, 1967, **4**, 115.
[266] W. Sibral and L. Schmid, *Tetrahedron Letters*, 1967, 4239.
[267] D. Trimnell, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **5**, 166.

hydrolysis, gave 1,4-anhydro-D-mannitol.[268] The product readily lost carbon dioxide and carbon disulphide to give bis-(2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl) disulphide which was independently synthesised from the corresponding ethyl xanthate by treatment with sodium methoxide in methanol followed by iodine. Both dimeric derivatives show n.m.r. singlets for the anomeric protons and were consequently assigned α-configurations, but the ethyl xanthate showed $J_{1,2}$ 3·5 c./sec. and was considered, without rationalisation, to be a β-derivative. Alternatively, xanthation of 2,3:5,6-di-*O*-isopropylidene-D-mannitol followed by coupling with iodine gave the corresponding bis-(*O*-thiocarbonyl) disulphide which on standing in pyridine decomposed to sulphur, carbon disulphide, di-*O*-isopropylidene-D-mannitol, and the orthocarbonate (101).[268]

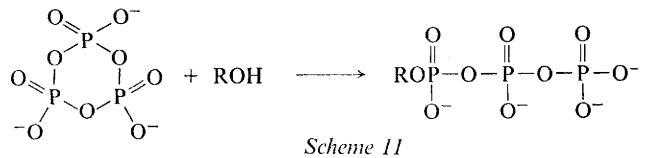


(101)

The xanthate ester group is a satisfactory chromophore for o.r.d. studies and it has now been shown that methyl, ethyl, propyl, butyl, cyclohexyl, and benzyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl xanthates exhibit positive Cotton effects, the molecular amplitudes of which decrease with increase in the size of the alkyl group.[269]

Phosphates

A new direct synthesis of monoalkyl phosphates has been reported which should be readily applicable in the carbohydrate field. Borneol, as a model substrate, was phosphorylated in acetonitrile solution with phosphorous acid, mercuric chloride as oxidant, and triethylamine, and the bornyl dihydrogen phosphate was isolated in 47% yield as the monoanilinium salt.[270] A further reaction of general significance is that shown by trimetaphosphate (as the sodium salt) which reacted with alcohols in aqueous alkaline solution to give alkyl triphosphates (Scheme 11). Ethylene glycol,



Scheme 11

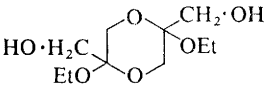
[268] W. M. Doane, B. S. Shasha, C. R. Russell, and C. E. Rist, *J. Org. Chem.*, 1967, **32**, 1080.

[269] Y. Tsuzuki, K. Tanabe, M. Akagi, and S. Tejima, *Bull. Chem. Soc. Japan*, 1967, **40**, 628.

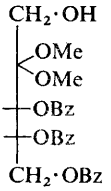
[270] T. Obata and T. Mukaiyama, *J. Org. Chem.*, 1967, **32**, 1063.

however, gave 2-hydroxyethyl monophosphate (presumably as a result of intramolecular base-catalysed hydrolysis of the triphosphate); preliminary experiments with carbohydrates showed that mono- and tri-phosphates were produced.[271]

Sugar Phosphates.—Further syntheses of specific phosphates have been reported. A four-step process starting from the cyclic acetal (102) has been used to obtain dihydroxyacetone phosphate in 27% overall yield.[272] Stewart and Ballou, continuing their investigation of the synthesis of short-chain carbohydrate phosphates, have prepared D-*erythro*-pentulose



(102)



(103)

1-phosphate, a compound closely related to many metabolic intermediates but which has not itself been found in nature. 3,4,5-Tri-*O*-benzoyl-D-*erythro*-pentulose was prepared *via* the 1-deoxy-diazo-compound and was converted to the dimethyl acetal (103). Phosphorylation and removal of the protecting groups gave the 1-ester which was found to be inactive as a substrate for D-ribulose 1,5-diphosphate carboxylase (the enzyme controlling carbon fixation at the outset of photosynthesis). It was observed that for the acetalation of the carbonyl group of a ketose, there must be present a hydroxy-group at a neighbouring carbon.[273]

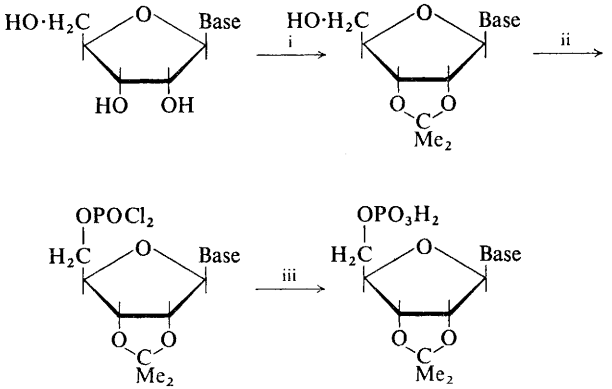
For requirements in polysaccharide biosynthesis studies, hexosyl phosphates with the L-*ido*- and D-*manno*-configurations have been synthesised. Penta-*O*-acetyl-L-idopyranose was phosphorylated directly with anhydrous phosphoric acid to give, after deacetylation, a mixture of the anomeric 1-phosphates from which the α-compound was separated as a salt. Similar procedures in the *manno*-series gave only the α-phosphate and this difference was discussed in terms of conformational analysis. Oxidation of these esters with oxygen over a platinum catalyst gave the glycosyluronic acid phosphates.[274] The same phosphorylation procedure has been adopted to obtain α-D-glucopyranose 1,6-diphosphate from the 6-phosphate in 25% yield *via* 1,2,3,4-tetra-*O*-acetyl-D-glucopyranosyl 6-phosphate,[275] and

[271] W. Feldmann, *Chem. Ber.*, 1967, **100**, 3850.
[272] R. L. Colbran, J. K. N. Jones, N. K. Matheson, and I. Rozema, *Carbohydrate Res.*, 1967, **4**, 355.
[273] J. C. Stewart and C. E. Ballou, *J. Org. Chem.*, 1967, **32**, 1065.
[274] P. Perchemlides, T. Osawa, E. A. Davidson, and R. W. Jeanloz, *Carbohydrate Res.*, 1967, **3**, 463.
[275] W. A. Kahn, T. Baldwin, U. Brodbeck, and K. E. Ebner, *Analyt. Biochem.*, 1967, **20**, 58.

sedoheptulose 7-phosphate has been built up from D-ribose 5-phosphate by application of the nitroethanol procedure.[276]

Attention has been given to the degradation of D-glucose 6-phosphate and amongst the products D-glucometasaccharinic acid 6-phosphate, D-fructose 6-phosphate, and D-glyceraldehyde 3-phosphate have been identified.[277] This alkaline-catalysed process was then compared with the anaerobic enzymic metabolism of the ester, and the two processes were shown to be strikingly similar which led to the interesting suggestion that metabolic pathways in chemical evolution were available before the enzymes required for their pursuit.[278]

Nucleoside Phosphates and Phosphites.—Two related, ingenious methods have been reported for the simple sequential protection of the 2′,3′-diol of ribonucleosides and the phosphorylation of the 5′-group. In the first phosphorous oxychloride in acetone was used to catalyse the formation of 2′,3′-*O*-isopropylidene derivatives which, on the addition of pyridine, were esterified at the primary site. Aqueous hydrolysis finally gave the 5′-phosphate (Scheme 12).[279]



Reagents: i, Me_2CO, $POCl_3$; ii, Pyr; iii, H_2O.

Scheme 12

In the second, β-cyanoethylphosphoric acid was used as the acid catalyst in the reaction of the nucleoside with trimethyl orthoformate to give 2′,3′-*O*-methoxymethylidene compounds, excess of orthoformate was removed, pyridine and DCC were added, and the cyanoethylphosphoric acid then became the phosphorylating agent; finally the protecting groups were removed by standard procedures.[280]

[276] B. A. McFadden, L. L. Barden, N. W. Rokke, M. Uyeda, and T. J. Siek, *Carbohydrate Res.*, 1967, **4**, 254.
[277] C. Degani and M. Halmann, *Israel J. Chem.*, 1967, **5**, 59P.
[278] C. Degani and M. Halmann, *Nature*, 1967, **216**, 1207.
[279] Y. Fujimoto and M. Naruse, *J. Pharm. Soc. Japan*, 1967, **87**, 270.
[280] J. L. Darlix, H. M. P. Fromageot, and P. Fromageot, *Biochem. Biophys. Acta*, 1967, **145**, 517.

Other specific 5′-nucleotides have been prepared with the aid of phenylboronic acid to protect the 2′- and 3′-positions: the method has been applied to obtain the 5′-phosphates of uridine and cytidine,[281] and the 5′-di- and tri-phosphates of adenosine and uridine.[282] During this work the effect of treating adenosine monophosphates with phenylboronic acid in DMF at elevated temperatures was investigated. A 2′,3′-cyclic phosphate and a 2′,3′-phenylboronate were formed, as well as adenosine itself; from a mixture of 2′- and 3′-monophosphates, the 3′-isomer was obtained suggesting that de-esterification occurred preferentially at the 2′-position.[283]

Di-(2,2,2-trichlorethyl)phosphorochloridate in pyridine has been shown to cause satisfactory phosphorylation of protected nucleosides; the trichloroethyl groups could be removed either with zinc–copper in DMF or with zinc in acetic acid.[284] In other work intended to throw light on prebiological nucleotide synthesis, it has been shown that several nucleosides were converted to mono-, di, tri-, and higher phosphates with polyphosphoric acid at temperatures in the range 0–22°. Deoxyadenosine and deoxyguanosine were, however, found to resist phosphorylation under these conditions.[285] Other reported phosphorylations of nucleosides have involved the use of phosphoryl chloride in trialkyl phosphate solvents,[286] and of the standard cyanoethyl phosphate—DCC method.[287] A search for cytotoxic materials has led to the synthesis of bis(pyrimidine nucleoside) phosphates, prepared with *p*-nitrophenylphosphorodichloridate as esterifying agent.[288]

The trichloroethyl group, mentioned above[284] in the synthesis of nucleoside phosphates, can also be used in their protection since, in the presence of hydroxy-groups, 2,2,2-trichloroethanol condensed specifically with monophosphates under the influence of DCC. The hydroxy-groups could then be esterified and the phosphate regenerated, so that a route was opened for the synthesis of oligonucleotides.[289]

A detailed study has been made of the kinetics of the nonenzymic hydrolysis of nucleoside 2′,3′-phosphates,[290] and the enzymic phosphorylation of a large number of adenosine derivatives (including 3′-*C*-methyladenosine) has been examined and the structural modifications which permit phosphorylation have been summarised.[291]

[281] A. M. Yurkevich, L. S. Varshavskaya, I. I. Kolodkina, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1967, **37**, 2002.
[282] I. I. Kolodkina, L. S. Varshavskaya, A. M. Yurkevich, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1967, **37**, 1996.
[283] A. M. Yurkevich, V. I. Borodulina-Shvets, I. I. Kolodkina, and N. A. Preobrazhenzkii, *Zhur. obshchei Khim.*, 1967, **37**, 2176.
[284] F. Eckstein and K. H. Scheit, *Angew. Chem. Internat. Edn.*, 1967, **6**, 362,
[285] T. V. Waehneldt and S. W. Fox, *Biochem. Biophys. Acta*, 1967, **134**, 1.
[286] M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, 1967, 5065.
[287] J. Smrt, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3958.
[288] J. A. Montgomery and H. J. Thomas, *J. Medicin. Chem.*, 1967, **10**, 1163.
[289] F. Eckstein, *Chem. Ber.*, 1967, **100**, 2228; 2236.
[290] H. I. Abrash, C.-C. S. Cheung, and J. C. Davis, *Biochemistry*, 1967, **6**, 1298.
[291] H. T. Shigeura and S. D. Sampson, *Nature*, 1967, **215**, 419.

Nucleoside 5′-phosphites have been prepared with 2′,3′-*O*-ethoxymethylene groups as protecting functions. The reaction of triphenylphosphite in DMF required the presence of a base such as triethylamine, otherwise the orthoester ring opened and 2′- and 3′-phosphites were also formed; the method offers perhaps the best means of preparing nucleoside 5′-phosphites.[292]

Sulphates

Warnings have been given against the use of i.r. spectral methods for the assignment of positions of sulphate esters on pyranoid compounds. It was previously claimed that absorptions at 850, 830, and 820 cm.$^{-1}$ were diagnostic of C—O—S bonds in compounds having secondary axial, secondary equatorial, and primary equatorial ester groups respectively, but it has now been reported that the physical state of the specimen governs, to a large extent, the precise position of this absorption band.[293] Methyl α-D-galactopyranoside 2,3-disulphate, barium salt, for example, showed maximum absorption at 833 cm.$^{-1}$ when measured in a mull, but at 855 cm.$^{-1}$ when measured as a film.

Arylsulphuryl chlorides act as suitable, mild sulphating agents and have been used in highly specific monosulphations of mono-[294] and oligosaccharides.[295] In these and other studies on di- and oligosaccharide sulphates,[296] the sites of esterification were determined by methylation techniques.

A variety of sulphates has been investigated in the course of biochemical or pharmacological studies. D-Galactose 6-[^{35}S]sulphate and 3-[^{35}S]sulphate were prepared by standard methods from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-D-galactopyranose, respectively, using the pyridine–[$^{35}SO_3$] complex, and were used to facilitate the characterisation of galactose sulphates obtained after sulphation and hydrolysis of cattle brain cerebrosides.[297] Mono-, di-, and oligosaccharide sulphates showed pepsin inhibition and anti-ulcerogenic properties comparable with those of sulphated polysaccharides, and so the biological properties of the polymers were ascribed to the presence of the ester function rather than to a macromolecular property.[298, 299] Treatment of the sodium salt of sucrose sulphate with

[292] A. Holý, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3064.
[293] J. R. Turvey, D. M. Bowker, and M. J. Harris, *Chem. and Ind.*, 1967, 2081.
[294] K. Takiura and S. Honda, *J. Pharm. Soc. Japan*, 1967, **87**, 1248.
[295] K. Takiura and S. Honda, *J. Pharm. Soc. Japan*, 1967, **87**, 1256.
[296] K. Takiura and S. Honda, *J. Pharm. Soc. Japan*, 1967, **87**, 1052.
[297] H. Jatzkewitz and G. Nowoczek, *Chem. Ber.*, 1967, **100**, 1667.
[298] M. Namekata, A. Matsuo, A. Momose, and M. Takagi, *J. Pharm. Soc. Japan*, 1967, **87**, 376.
[299] M. Namekata, N. Sakamoto, Y. Yokoyama, and M. Takagi, *J. Pharm. Soc. Japan*, 1967, **87**, 778.

aluminium dihydroxychloride gave a complex which had greater anti-ulcerogenic activity than did the sodium salt itself.[300]

Sulphate esters of 5-iododeoxyuridine and 5-iododeoxycytidine have been prepared and were found to be inactive as growth inhibitors of mastocytoma cells. The pyridine–sulphur trioxide complex gave mixtures of 3′- and 5′-esters and 3′,5′-diesters which were identified by the specific synthesis of the 3′-esters using 5′-tritylated nucleosides. The diesters predominated under these conditions, higher yields of mono-substituted compounds being obtained in sulphations with chlorosulphonic acid.[301]

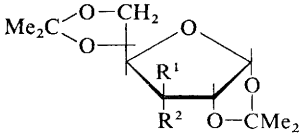
Chlorosulphates produced on treatment of D-ribose and its methyl β-pyranoside with sulphuryl chloride are mentioned on p. 86.

Sulphonates*

Appreciable interest remains in the sulphonate esters, particularly in their nucleophilic displacement reactions which have proved to be widely applicable in synthetic and structural work. A new synthesis of cyclic carbonates from sulphonyl esters has already been noted.[258]

Synthesis.—Two reports of partial toluene-*p*-sulphonylation (other than at a primary hydroxy-group) have appeared. Methyl 6-*O*-trityl-β-D-galacto-furanoside gave the 5-; 3-; 2-; 2,5-esters in the ratio of 11 : 6 : 7 : 12; the products were characterised by methylation followed by removal of the ester groups.[160] Partial esterification of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside gave the 2-*O*-toluene-*p*-sulphonyl derivative.[246]

Displacement Reactions without Participation.—Several direct displacements have been described. Displacement of the sulphonyloxy-group from 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose is well known for its difficulty; it has now been shown that reactions of the C-3-epimer, the *allo*-analogue, occur readily. Treatment of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (104) with sodium benzoate in DMF gave[302] the 3-*O*-benzoyl-*gluco*-compound (105) and a



(104) $R^1 = H$, $R^2 = TsO$
(105) $R^1 = BzO$, $R^2 = H$
(106) $R^1 = N_3$, $R^2 = H$

similar reaction with sodium azide[302, 303] gave the corresponding azide (106) which could be converted into the acetamido-compound. The displacement with fluoride ion has also been reported (see p. 85).

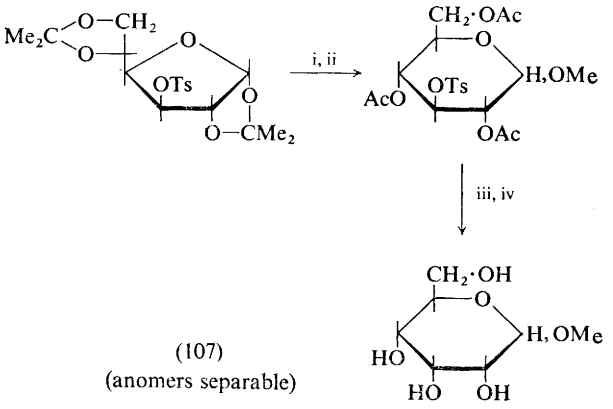
[300] M. Namekata, T. Tanaka, N. Sakamoto, and K. Moro, *J. Pharm. Soc. Japan*, 1967, **87**, 889.
[301] P. K. Chang, L. J. Sciarini, and J. W. Cramer, *J. Medicin. Chem.*, 1967, **10**, 733.
[302] D. T. Williams and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 7.
[303] J. S. Brimacombe, J. G. H. Bryan, A. Husain, M. Stacey, and M. S.Tolley, *Carbohydrate Res.*, 1967, **3**, 318.

* See also Section 21, pp. 153–160.

Benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-methanesulphonyl-β-D-glucopyranoside has been converted into benzyl 2-acetamido-2-deoxy-β-D-galactopyranoside by treatment with glacial acetic acid, potassium acetate, and a little water, followed by deacetylation.[304] Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methanesulphonyl-α-D-allopyranoside gave the corresponding 3-azido-3-deoxy-*gluco*-compound on treatment with sodium azide in DMF.[305]

Specific stereochemical inversions can be useful in the characterisation of partially substituted compounds; for example, the dibenzoate obtained on partial benzoylation of methyl 6-deoxy-α-L-galactopyranoside.[245] Since the derived methanesulphonate reacted smoothly with sodium benzoate in DMF–DMSO to give methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-glucopyranoside, the free hydroxy-group in the initial dibenzoate was located at C-4; analogous reaction of the sulphonate with sodium azide offered a route to 4-amino-4,6-dideoxy-L-glucose. However, the sulphonyloxy-group in methyl 2,3-di-*O*-benzoyl-6-deoxy-4-*O*-toluene-*p*-sulphonyl-α-L-mannopyranoside was resistant to similar nucleophilic displacements. In like fashion, the partially substituted derivatives obtained in benzoylation studies on pentopyranosides were characterised by sulphonylations followed by sulphonyloxy-displacements.[247]

A convenient synthesis of methyl α- and β-D-allopyranosides and thence of D-allose from 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose has been developed by the route shown in Scheme 13.[306] The β-anomer of (107) reacted much more rapidly than the α-anomer. This



(107)
(anomers separable)

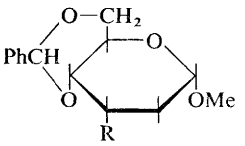
Reagents: i, MeOH–HCl; ii, Ac_2O–NaOAc; iii, $NaOB_z$–DMF; iv, NaOMe–MeOH.

Scheme 13

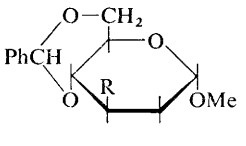
[304] P. H. Gross, F. Du Bois, and R. W. Jeanloz, *Carbohydrate Res.*, 1967, **4**, 244.
[305] Y. Ali and A. C. Richardson, *Chem. Comm.*, 1967, 554.
[306] R. Ahluwahlia, S. J. Angyal, and M. H. Randall, *Carbohydrate Res.*, 1967, **4**, 478.

was of interest since it had been reported (N. A. Hughes *et al.*, *J. Chem. Soc.*, 1965, 2236) that 1,2,4,6-tetra-*O*-benzoyl-3-*O*-toluene-*p*-sulphonyl-β-D-glucopyranose underwent displacement with sodium benzoate whereas the α-anomer did not; this was attributed by those authors to hindrance by the axial C-1 group in the α-anomer to the rearward approach to C-3 by the nucleophile. It was suggested that in the present case,[306] the α-anomer reacted because of the smaller size of the methoxy-group compared with benzoyloxy. For the synthesis of D-allose, the mixture of anomers (107) can be used, and the resulting mixture of methyl D-allopyranosides hydrolysed to the free sugar.

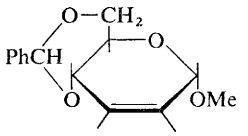
A comparison of the nucleophilic displacement of the epimeric methanesulphonates (108) and (111) with various nitrogen-containing nucleophiles has been made.[307] With the axial ester (108), sodium azide in DMF gave the azido-sugar (112) as the sole product, ammonia gave the amino-sugar



(108) R = OMs
(109) R = N_3
(110) R = NH_2



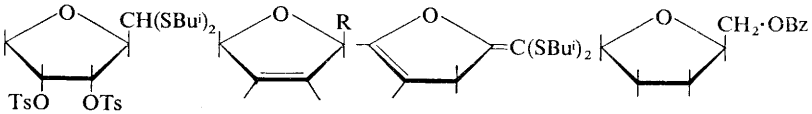
(111) R = OMs
(112) R = N_3
(113) R = NH_2



(114)

(113) as its methanesulphonate salt (20%), together with the unsaturated sugar (114) (80%), and with hydrazine displacement predominated to give (after reduction) (113) (71%) accompanied by some elimination to (114) (27%). For the equatorial ester (111), no elimination was observed, as might be expected; ammonia gave only starting compound, whereas azide ion and hydrazine gave the expected products (109) and (110), the latter after reduction.

Treatment of 2,5-anhydro-3,4-di-*O*-toluene-*p*-sulphonyl-D-ribose di-isobutyl dithioacetal (115) with sodium iodide and zinc in DMF gave 2,5-dihydro-2(*R*)-formylfuran di-isobutyl dithioacetal (116).[308] However, the *xylo*-isomer of (115) (*trans*-sulphonyloxy-groups) gave the diene (118). The *xylo*-dimethyl acetal analogue did not react under these conditions. The dithioacetal (115) has been converted by standard sequences into the



(115)

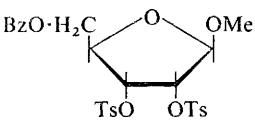
(116) R = $CH(SBu^i)_2$
(117) R = $CH_2 \cdot OBz$

(118)

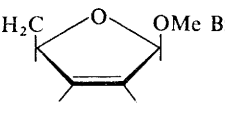
(119)

[307] J. Kovář, V. Dienstbierová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2498.
[308] J. Defaye and J. Hildesheim, *Bull. Soc. chim. France*, 1967, 940.

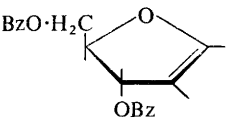
2,5-anhydro-1-*O*-benzoyl-D-ribitol derivative which, with sodium iodide and zinc in DMF, gave 2(*R*)-benzoyloxymethyl-2,5-dihydrofuran (117), which was reduced to the optically active tetrahydrofuran derivative (119).[309a] The same olefin-forming reaction has been applied to methyl 5-*O*-benzoyl-2,3-di-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside (120) (prepared by selective displacement of the primary sulphonyloxy-group from methyl 2,3,5-tri-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside by benzoate ion).[309b] The product, methyl 5-*O*-benzoyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranoside (121) was identical with the product of known anomeric



(120)



(121)

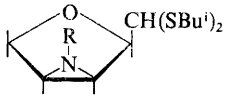


(122)

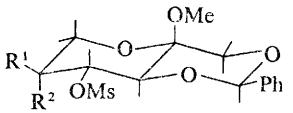
configuration that resulted from the attack of methanol on (122) (H. G. Fletcher, jun., *et al.*, *J. Org. Chem.*, 1963, **28**, 435). Reduction of (121) gave the corresponding 2,3-dideoxyribofuranoside.

Preferential displacement of the primary group in methyl 2,3,5-tri-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside also occurred with sodium azide in DMF if brief reaction times were used.[310] Selective displacement of one of the sulphonyloxy-groups in the *cis*-di-*O*-toluene-*p*-sulphonyl derivative (115) occurs with sodium azide in DMF.[311] No proof of the site of reaction was given but it was assumed to have occurred at the less hindered C-4 atom. The *trans*-configuration of the product was, however, demonstrated by its conversion to the epimino-derivatives (123).

Selective displacement of the 5-methanesulphonyloxy-group has been shown to occur in methyl 1,3-*O*-benzylidene-4,5-di-*O*-methanesulphonyl-α-L-sorbopyranoside (124) with azide or benzoate ion to give the β-D-fructopyranoside compounds (125);[312] the same selective displacement occurred



(123)
R = Ac or $C_6H_3(NO_2)_2$



(124) R^1 = OMs, R^2 = H
(125) R^1 = H, R^2 = N_3 or OBz
(126) R^1 = H, R^2 = OMs
(127) R^1 = N_3, R^2 = H

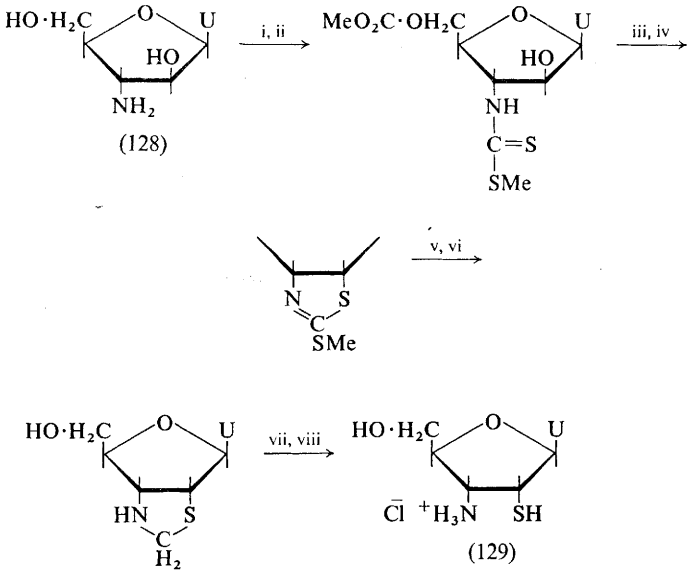
[309a] J. Cleophax and S. D. Gero, *Bull. Soc. chim. France*, 1967, 1441.
[309b] J. Hildesheim, J. Cleophax, and S. D. Gero, *Tetrahedron Letters*, 1967, 1685.
[310] J. Hildesheim, J. Cleophax, S. D. Gero, and R. D. Guthrie, *Tetrahedron Letters*, 1967, 5013.
[311] J. Cleophax, S. D. Gero, and R. D. Guthrie, *Tetrahedron Letters*, 1967, 567.
[312] D. Murphy, *J. Chem. Soc.* (*C*), 1967, 1732.

in the β-D-*fructo*-disulphonyl ester (126) to give the 5-azido-α-L-sorbopyranoside (127). In both (124) and (126), C-5 is much less hindered towards S_N2 attack than is C-4.

It is of particular interest to note that the mechanism of reductive removal of a primary tosylate apparently is solvent dependent, and that S—O bond cleavage occurs mainly in tetrahydrofuran whereas C—O fission predominates in ether–benzene.[159]

Displacement Reactions with Participation.—A comprehensive review on such reactions has been published.[313]

(a) *Sulphur functions.* The use of the complex neighbouring group approach has been used in the synthesis of 3′-amino-3′-deoxy-2′-thio-uridine as its hydrochloride (129) from 1-(3′-amino-3′-deoxy-β-D-arabinofuranosyl)uracil (128) by the route shown in Scheme 14.[314] A thionobenzoate group has



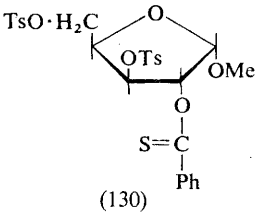
Reagents: i, CS_2–MeI; ii, MeO·COCl; iii, MsCl–pyr; iv, pyr; v, MeONa–MeOH; vi, Al–Hg; vii, aq. $HgCl_2$; viii, H_2S–MeOH.

Scheme 14

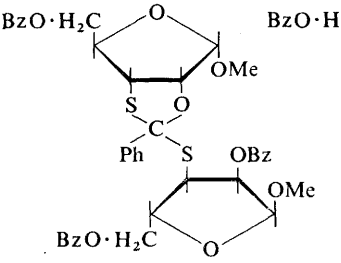
been used also in a pentofuranose system to obtain a thiol group at C-3 in D-ribose derivatives.[315] Treatment of methyl 2-*O*-thionobenzoyl-3,5-di-*O*-toluene-*p*-sulphonyl-α-D-xylofuranoside (130) with sodium benzoate in DMF gave the dimer (131), formed from the ion (132). Compound (131) after mild hydrolysis, benzoylation, and acetolysis gave (133) which was used in the synthesis of 3′-thioadenosine.

[313] L. Goodman, *Adv. Carbohydrate Chem.*, 1967, **22**, 109.
[314] T. Sekiya and T. Ukita, *Chem. and Pharm. Bull (Japan)*, 1967, **542**, 1503.
[315] E. M. Acton, K. J. Ryan, and L. Goodman, *J. Amer. Chem. Soc.*, 1967, **89**, 467.

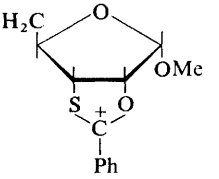
Reaction of 3,4,6-tri-*O*-acetyl-2-*O*-methanesulphonyl-β-D-glucopyranosyl *NN*-dimethyldithiocarbamate (134) with potassium acetate in ethanolic acetone gave an unsaturated product, shown by n.m.r. and chemical methods to be the 2-(*NN*-dimethyldithiocarbamoyl) derivative (135), necessitating the formulation of an ion such as (136) as an intermediate.[316]



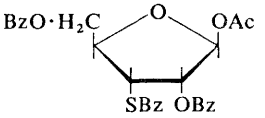
(130)



(131)

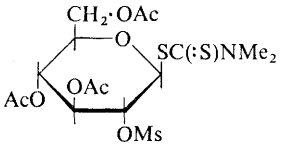


(132)

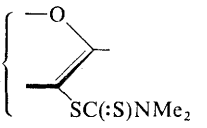


(133)

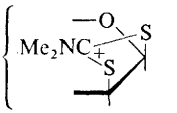
Reaction of (134) with potassium thioacetate in the same solvent, however, gave a product containing the SAc group, formulated as the 2-thioacetate (137), that is, resulting from direct S_N2 displacement of the sulphonyloxy-group at C-2. In the Reviewers' opinion, that the displacement took place



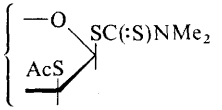
(134)



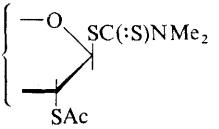
(135)



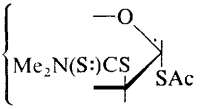
(136)



(137)



(138)

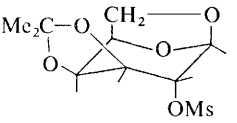


(139)

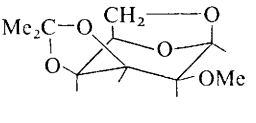
in only 30 min. and because of the solvents used, neighbouring-group participation *via* ion (136) most probably occurred so that the product would be either (138) or (139). Since it was reported to show a $J_{1,2}$ value of 2·5 c./sec., structure (139) appears the more probable.

[316] S. Ishiguro and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1478.

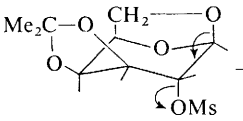
(b) *Oxygen functions.* In 1959 it was reported (Kent *et al.*, *Proc. Chem. Soc.*, 1959, 187) that solvolysis of 1,6-anhydro-3,4-*O*-isopropylidene-2-*O*-methanesulphonyl-β-D-galactopyranose (140) in methanolic potassium hydrogen fluoride gave 1,6-anhydro-3,4-*O*-isopropylidene-2-*O*-methyl-β-D-talopyranose (141). In a preliminary report [317] it has been pointed out that



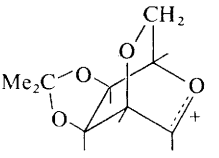
(140)

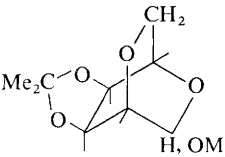


(141)



(142)





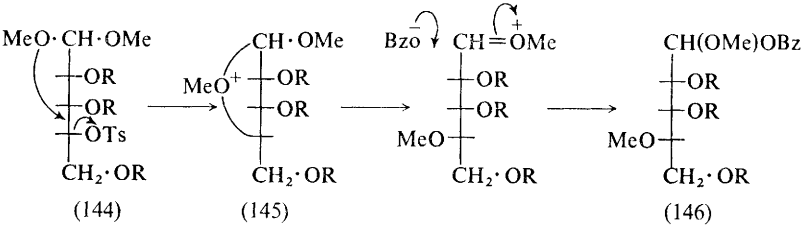
(143)

this is an unlikely result, because of the steric hindrance to direct S_N2 displacement of the methanesulphonyloxy-group, and the product has now been shown to be a mixture of anomers of methyl 2,6-anhydro-3,4-*O*-isopropylidene-D-talopyranoside (143), formed by intramolecular participation of the C-1—O bond, as shown in (142). The major product was probably the β-anomer. Compound (143) was characterised by conversion into the known 1,5-anhydro-D-altritol (2,6-anhydro-D-talitol).

Two other papers from Hughes' group have dealt with neighbouring-group participation in sulphonyl displacements, both involving acyclic carbohydrate derivatives.[318, 319] One paper reports the full details of the interesting 1 → 4-methoxy-migration that occurred when 2,3,5-tri-*O*-benzyl-4-*O*-toluene-*p*-sulphonyl-D-ribose dimethyl acetal (144) was treated with tetra-*n*-butylammonium benzoate in *N*-methylpyrrolidone.[318] The

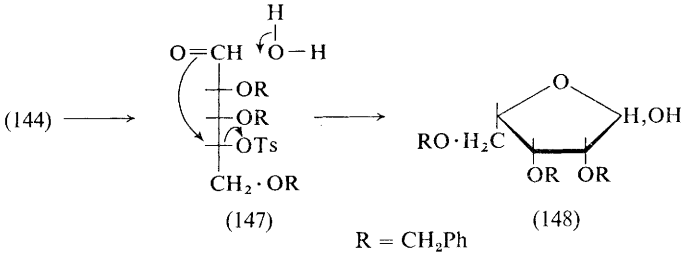
[317] N. A. Hughes, *Chem. Comm.*, 1967, 1072.
[318] N. A. Hughes and P. R. H. Speakman, *J. Chem. Soc.* (*C*), 1967, 1182.
[319] N. A. Hughes and P. R. H. Speakman, *J. Chem. Soc.* (*C*), 1967, 1186.

product was not the expected 4-*O*-benzoyl compound that would have resulted from direct S_N2 displacement, but was 1-*O*-benzoyl-2,3,5-tri-*O*-benzyl-4-*O*-methyl-L-lyxose methyl hemiacetal (146); the mechanism postulated is shown in Scheme 15. Alternatively, direct attack by benzoate



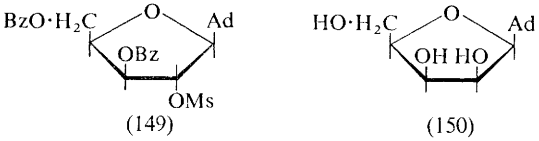
Scheme 15: R = CH_2Ph

ion could have occurred on the ion (145). Acid hydrolysis of the acetal (144) gave 2,3,5-tri-*O*-benzyl-4-*O*-toluene-*p*-sulphonyl-*aldehydo*-D-ribose (147) and 2,3,5-tri-*O*-benzyl-L-lyxofuranose (148); the latter was the sole product after prolonged hydrolysis.[319] Treatment of (147) with methanolic sodium methoxide gave the methyl glycoside of (148). The formation of the



lyxo-products in the above reactions was postulated to proceed *via* participation of the aldehyde group in the displacement of the sulphonyloxy-group, as shown.

Neighbouring-group influences can give rise to mixed products and can be controlled to some degree to enhance yields of particular isomers.



Treatment of 9-(3,5-di-*O*-benzoyl-2-*O*-methanesulphonyl-β-D-xylofuranosyl)adenine (149) with sodium benzoate in DMF followed by debenzoylation gave a product containing approximately equal quantities of D-*xylo*-, D-*arabino*-, and D-*lyxo*-nucleosides. In an effort to increase the yield of the

lyxo-isomer, the authors then used sodium fluoride in DMF with beneficial results.[320] Proportions of isomers produced under various conditions are shown in Table 2. The *lyxo*-nucleoside (150), the last of the series of

Table 2

	Product proportions		
Reagent	*xylo*	*arabino*	*lyxo*
NaOBz–anhydrous DMF	0·7	0·8	1·0
NaOBz–DMF–10% H_2O	0·9	1·6	1·0
NaOAc–anhydrous DMF	1·0	1·0	1·0
NaOAc–DMF–10% H_2O	1·0	1·7	1·0
$NaHCO_3$–anhydrous DMF	3·0	1·0	1·0
$NaHCO_3$–DMF–10% H_2O	4·2	7·0	1·0
NaF–anhydrous DMF	0·3	0·4	1·0

β-D-pentofuranosyladenines to be prepared, was isolated by ion-exchange chromatography and was converted by a standard series of reactions to 9-(β-D-lyxofuranosyl)-9*H*-purine-6-thiol. Treatment of (150) with acetone or benzaldehyde in the presence of acid catalysts gave mixtures of 2′,3′- and 3′,5′-cyclic acetals. The isopropylidene compounds were characterised by observation of the methyl proton chemical shifts and by noting that the 2′,3′-isomer on methanesulphonylation and acid hydrolysis gave a 3,5′-cyclonucleoside; the benzylidene derivatives were assigned structures on the basis of comparisons of their n.m.r. spectra with those of the isopropylidene analogues.[320]

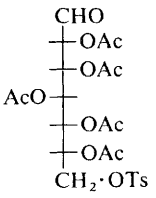
A new type of assistance in sulphonyloxy-group displacements, namely by a non-vicinal hydroxy-group, has been described.[321] Displacement of the toluene-*p*-sulphonyloxy-group by thiocyanate ion in DMF occurred in 1,2-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-xylofuranose, but not in the corresponding 5-deoxy-derivative. The product was characterised by conversion to the corresponding 3-deoxy-derivative. It was suggested that the neighbouring hydroxy-group on C-5 assisted displacement. Similar results were reported for D-glucofuranose compounds.

Participation of a benzyloxy-group has been reported.[322] Solvolysis of methyl 2,3-di-*O*-benzyl-6-*O*-methanesulphonyl-β-D-galactopyranoside gave methyl 3,6-anhydro-2-*O*-benzyl-β-D-galactopyranoside. Methyl 2,3-di-*O*-benzyl-4-*O*-methanesulphonyl-β-D-galactopyranoside was unaffected by the same reaction conditions.

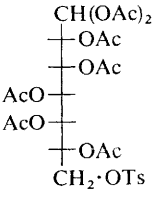
Penta-*O*-acetyl-7-*O*-toluene-*p*-sulphonyl-*aldehydo*-D-*glycero*-D-*gulo*-heptose (151) and hepta-*O*-acetyl-7-*O*-toluene-*p*-sulphonyl-*aldehydo*-D-*glycero*-L-*manno*-heptose (152) have been subjected to total racemisation with zinc chloride in acetic anhydride. Numerous isomers were isolated from the resulting mixture of isomeric heptoses. Isomerisation of (151) gave an anhydro-compound to which the structure 1,6-anhydro-L-*glycero*-β-D-*gulo*-heptopyranose has been tentatively assigned.[323] Reaction of

[320] E. J. Reist, D. F. Calkins, and L. Goodman, *J. Org. Chem.*, 1967, **32**, 169.
[321] J. Defaye and J. Hildesheim, *Carbohydrate Res.*, 1967, **4**, 145.
[322] J. S. Brimacombe and O. A. Ching, *Carbohydrate Res.*, 1967, **5**, 239.
[323] F. Micheel, H. Pfetzing, and G. Pirke, *Carbohydrate Res.*, 1967, **3**, 283.

1,1,2,3,4,5-hexa-*O*-acetyl-6-*O*-toluene-*p*-sulphonyl-D-galactose under the same conditions gave similar results.[324]

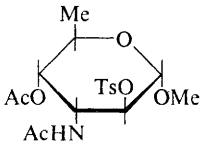


(151)

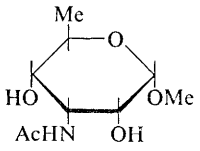


(152)

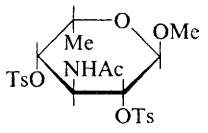
(c) *Nitrogen functions.* Solvolysis of methyl 3-acetamido-4-*O*-acetyl-3,6-dideoxy-2-*O*-toluene-*p*-sulphonyl-α-D-altropyranoside (153) has been shown to give the alloside derivative (154), by inversion at C-2.[234] Solvolysis of methyl 3-acetamido-3,6-dideoxy-2,4-di-*O*-toluene-*p*-sulphonyl-α-L-idopyranoside (155) gave the oxazoline (156) (52%). After treatment of the



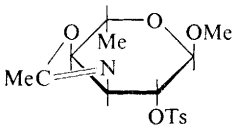
(153)



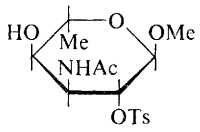
(154)



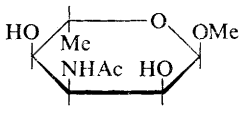
(155)



(156)



(157)



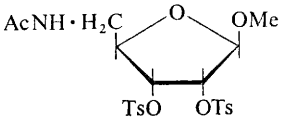
(158)

mother-liquor with aqueous ethanol, methyl 3-acetamido-3,6-dideoxy-2-*O*-toluene-*p*-sulphonyl-α-L-altropyranoside (157) (15%) and methyl 3-acetamido-3,6-dideoxy-α-L-allopyranoside (158) (12%) were isolated. Solvolysis of the 4-*O*-acetyl derivative of (157) gave (158).

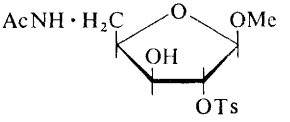
Two examples of participation by a non-vicinal acetamido-group have been reported.[310, 325] Treatment of methyl 5-acetamido-5-deoxy-2,3-di-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside (159) with sodium azide, benzoate, or fluoride in DMF gave methyl 5-acetamido-5-deoxy-2-*O*-toluene-*p*-sulphonyl-β-D-xylofuranoside (160).[310] Similarly, reaction of 5-acetamido-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-D-arabinofuranoside (161) with sodium benzoate in the same solvent gave the *lyxo*-derivative

[324] F. Micheel and E. Matzke, *Carbohydrate Res.*, 1967, **4**, 249.
[325] S. Hanessian, *J. Org. Chem.*, 1967, **32**, 163.

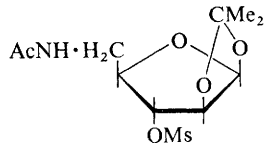
(162).[325] Both the above reactions occurred through participation of the acetamido-group, *via* an ion such as (163). The same displacement on the benzamido-analogue of (161) gave the phenyloxazoline (164) which could be isolated in high yield.[325] The 5-azido-analogue of (161) was much less reactive and suffered preferential demethanesulphonylation on treatment with the reagent.[325]



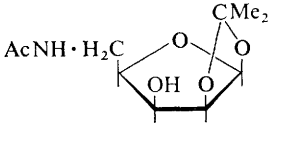
(159)



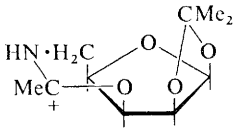
(160)



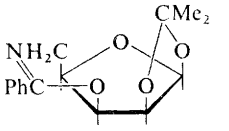
(161)



(162)

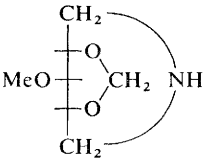


(163)



(164)

Treatment of 3-*O*-methyl-2,4-*O*-methylene-1,5-di-*O*-toluene-*p*-sulphonyl-xylitol with ammonia gave the cyclic product (165) which resulted from displacement of one sulphonyloxy-group, followed by intramolecular attack of the amino-group formed, on the other ester group.[326]

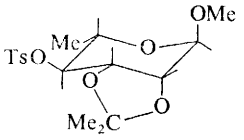


(165)

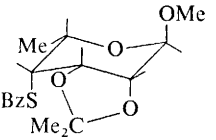
Displacement Reactions Accompanied by Rearrangement.—Further developments have followed the findings (Stevens *et al.*, *J. Amer. Chem. Soc.*, 1966, **88**, 2073; Hanessian, *Chem. Comm.*, 1966, 796) that rearrangement may accompany attempted displacements of sulphonyloxy-groups from the

[326] A. N. Anikeeva, L. G. Revelskaya, N. A. Khrenova, and S. N. Danilov, *Zhur. obshchei Khim.*, 1967, **37**, 997.

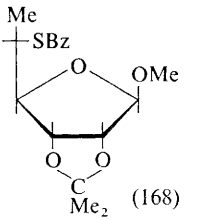
C-4-position. These observations led Stevens' group to re-investigate [327] the reported synthesis (Owen *et al.*, *J. Chem. Soc.*, 1966, 1291) of the '6-deoxy-4-thio-L-talopyranose' derivative (167) by attack of thiobenzoate ion on methyl 2,3-*O*-isopropylidene-4-*O*-toluene-*p*-sulphonyl-α-L-rhamnopyranoside (166), and to show that the product was, in fact, the 6-deoxy-L-*talo*-5-thiobenzoate (168). These findings then led Owen [328] to re-investigate



(166)



(167)



(168)

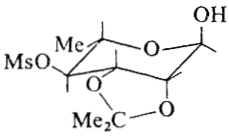
some of his own reported studies on the displacement reactions of some 4-*O*-toluene-*p*-sulphonyl-D-galactopyranoside derivatives, and he was able to show that, in this case, the structures originally assigned were correct. He further suggested [328] that rearrangement occurs in the system (166) because of the *trans*-antiparallel arrangement of the C-5—O and the C-4—OTs bonds and that a C-4 to C-5 migration occurs, followed by a normal S_N2 displacement at C-5. Such a reaction path would be of too high energy in the galactoside case, because of the conformation required for such a migration. It has been shown [329] that rearrangement did not occur when methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methanesulphonyl-α-D-galactopyranoside was treated with thiocyanate ion in DMF; the product had the 4-deoxy-4-thiocyano-D-*gluco*-structure.

Work with nitrogen-containing nucleophiles on the methanesulphonyl analogue of (166) has shown that the occurrence of the rearrangement is nucleophile-dependent. Reaction with sodium azide in DMF gave, by analogy with the reaction described above, the expected 5-azido-5-deoxy-talofuranose derivative.[330] However, reaction of (166) with hydrazine, followed by hydrogenation, gave methyl 4-amino-4-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside, *i.e.* no rearrangement had occurred.

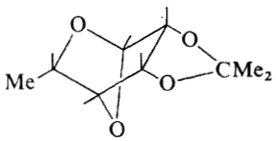
Methanolic sodium methoxide on 2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-rhamnopyranose (169) gave 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-talopyranose (1,5-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranose) (170).[331] It was not clear whether this reaction was a direct intramolecular displacement (which would have necessitated the β-anomer to react in a boat conformation) or whether it occurred by a ring-contraction

[327] C. L. Stevens, R. P. Glinski, G. E. Gutowski, and J. P. Dickerson, *Tetrahedron Letters*, 1967, 649.
[328] L. N. Owen, *Chem. Comm.*, 1967, 526.
[329] S. D. Gero and R. D. Guthrie, *J. Chem. Soc.* (*C*), 1967, 1761.
[330] J. Jarý, P. Novák, and Z. Ksandr, *Chem. and Ind.*, 1967, 1490.
[331] J. S. Brimacombe and L. C. N. Tucker, *Carbohydrate Res.*, 1967, **5**, 36.

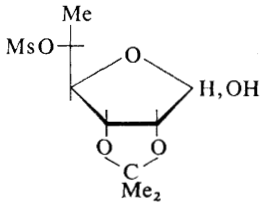
and migration of the type discussed above (reaction through the α-anomer). Compound (170) was also obtained by the action of sodium methoxide on 6-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulphonyl-D-allofuranose (171).[331]



(169)

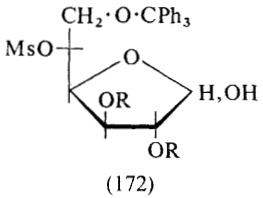


(170)



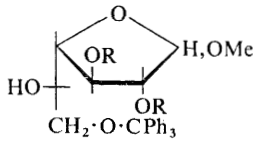
(171)

2,3-Di-*O*-benzyl-5-*O*-methanesulphonyl-6-*O*-trityl-D-glucofuranose (172) and methanolic sodium methoxide have been reported to give the rearranged product, methyl 2,3-di-*O*-benzyl-6-*O*-trityl-L-altrofuranoside (173).[332]



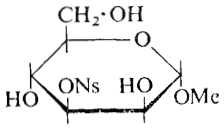
(172)

R = CH_2Ph

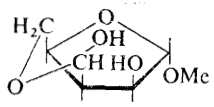


(173)

Reactions of Nitrobenzene-*p*-sulphonates.—Full details of the solvolysis of some nitrobenzene-*p*-sulphonyl esters have been reported.[183] Methyl 3-*O*-nitrobenzene-*p*-sulphonyl-α-D-mannopyranoside (174) gave methyl



(174)



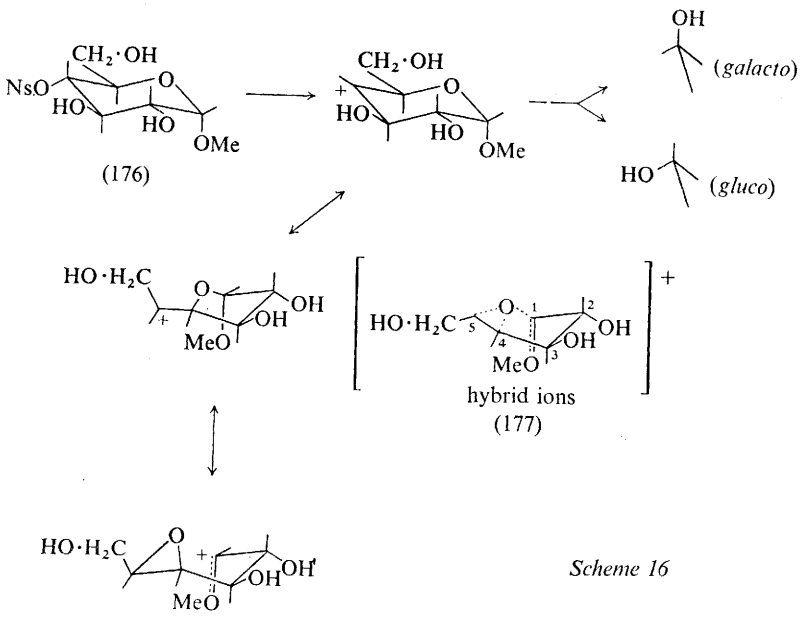
(175)

3-deoxy-3-formyl-α-D-lyxofuranoside (175), characterised as the corresponding lactone. The C-2-epimeric glucoside gave the 3-formyl *xylo*-analogue of (175). These products, the same as those resulting from the

[332] T. Iwashige and H. Saeki, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 132.

nitrous acid deamination of the appropriate methyl 3-amino-3-deoxyglycopyranosides, were formed by a ring-contraction involving the migration of the C-4—C-5 bond which is antiparallel to the C-3—O bond. The corresponding toluene-*p*-sulphonate did not undergo this reaction which was attributed to the superior character of the *p*-nitro-ester as a leaving group. Similar solvolysis of methyl 2-*O*-nitrobenzene-*p*-sulphonyl-α-D-glucopyranoside, followed by borohydride reduction, gave the same product as that from nitrous acid deamination of the corresponding 2-amino-2-deoxy-compound, followed by borohydride treatment.

A preliminary report [333] has appeared on the solvolysis of 4-*O*-nitrobenzene-*p*-sulphonates. The derivative (176) of methyl α-D-glucopyranoside gave a mixture of products: methyl α-D-glucopyranoside (50%), D-glucose (8%), methyl β-L-altrofuranoside (8%), and methyl α-D-galactopyranoside (8%), together with traces of other products. These results were rationalised by the sequence shown in Scheme 16. Attack on the ion (177) at C-5 with



Scheme 16

inversion would give the L-altrofuranoside; none of the C-5 epimer (methyl-α-D-galactofuranoside) could be detected. The D-glucose probably did not arise from direct hydrolysis of the glucoside, because the more labile altrofuranoside was found accompanied by no free sugar. Attack at C-1 of the ion (177) would give a 4,5-anhydro-D-galactose which could undergo intramolecular ring-opening to give D-glucose.

[333] P. W. Austin, J. G. Buchanan, and D. G. Large, *Chem. Comm.*, 1967, 418.

Nitrates

The synthesis of an α-D-glucopyranosyl nitrate using a Koenings–Knorr-type synthesis has already been mentioned.[67] Nitrate esters of 5-fluoro-2′-deoxyuridine have also been prepared and studied.[334] The symmetrical stretching frequencies of the nitrato-group in nitrate esters of 1,4:3,6-dianhydrohexitols have been shown to depend upon their *endo*- or *exo*-orientation. *endo*-Esters showed this absorption at 1282 ± 1 cm.$^{-1}$, whereas the *exo*-compounds absorbed at 1274 ± 1 cm.$^{-1}$; this observation offers a new means for determining structure and configuration of such compounds.[335] The same group of workers have suggested that circular dichroism of nitrate esters could be used for determining the configuration of optically active alcohols, since they found that the dichroism curves of mono- and di-nitrates of 1,4:3,6-dianhydro-derivatives of D-mannitol, L-iditol, and D-glucitol showed a weak positive maximum at 265 mμ and a second stronger band at 228 mμ, the sign of which was apparently dependent upon the configuration of the carbon bearing the nitrate group.[336]

Borates and Boronates

Borate anionic complexes are referred to elsewhere (see p. 149). Discrete esters have received only slight attention.

2′,3′-Phenylboronates of *ribo*-nucleosides have been used in 5′-phosphate synthesis,[281, 282] and the influence of phenylboronic acid on nucleoside monophosphates has also been noted.[283] 6-Substituted glucoses have been prepared using the 1,2:3,5-bisphenylboronate of the sugar[238]; the phenylboronate group has also been used as a blocking group for DMSO-based oxidations (see p. 138).

[334] R. Duschinsky and U. Eppenberger, *Tetrahedron Letters*, 1967, 5103.
[335] L. D. Hayward, D. J. Livingstone, M. Jackson, and V. M. Csizmadia, *Canad. J. Chem.*, 1967, **45**, 2191.
[336] L. D. Hayward and S. Claesson, *Chem. Comm.*, 1967, 302.

7
Halogenated Sugars

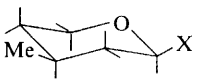
Glycosyl Halides

A new method of preparing glycofuranosyl chlorides has made use of the availability of furanosyl ring systems in aldonolactones and promises to have appreciable applicability in furanoside syntheses generally. It was illustrated by the synthesis of a mannofuranosyl nucleoside; reduction of 2,3:5,6-di-*O*-isopropylidene-D-mannono-γ-lactone with di-isopinocamphenyl borane gave the mannofuranose derivative in 90% yield and triphenylphosphine and carbon tetrachloride efficiently converted the free sugar to the glycosyl chloride, which could be treated directly with chloromercuri-6-benzamidopurine to give, after removal of the blocking group, 9-(D-mannofuranosyl)-adenine.[337] An additional, but less novel, method of obtaining glycosyl halides consisted of treating aldose peracetates having *trans*-1,2 related groups with hydrogen chloride in phosphorus trichloride. *trans*-1,2-Glycosyl chlorides were isolated from hexose derivatives indicating that C-2-acetoxy-participation was occurring, and this offers means for preparing thermodynamically unstable products in some instances (glucose and galactose, in particular); only pyranosyl acetates were investigated.[338] Other workers have found that treatment of tetra-*O*-acetyl-β-D-xylopyranose with aluminium chloride in cold chloroform solution was the best method of preparing tri-*O*-acetyl-β-D-xylopyranosyl chloride.[339]

The anomeric effect is particularly significant in the glycosyl halides with their strongly electronegative C-1 groups, and it is of pre-eminent significance in determining positions of anomeric equilibria and conformations. Following their work on the quantitative assessment of the anomeric effect within C-1-acetoxy and C-1-methoxy pyranoid model systems (*ca.* 1·3 kcal./mole for nonpolar solvents), Anderson and Sepp have measured the corresponding values for chloro, bromo, and iodo groups using 2-halogeno-tetrahydropyrans and 4-methyl analogues.[340] The best information was obtained by studying the equilibration of the

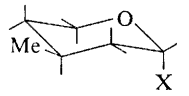
[337] J. B. Lee and T. J. Nolan, *Tetrahedron*, 1967, **23**, 2789.
[338] J. De Pascual Teresa and F. Hortal, *Anales real Soc. espaň. Fis. Quim.*, 1967, *B*, **63**, 221.
[339] C. V. Holland, D. Horton, and J. S. Jewell, *J. Org. Chem.*, 1967, **32**, 1818.
[340] C. B. Anderson and D. T. Sepp, *J. Org. Chem.*, 1967, **32**, 607.

conformationally homogeneous *cis*- and *trans*-2-halo-4-methyl-tetrahydropyrans (178) and (179). In carbon tetrachloride solutions the *trans*-isomers were heavily favoured, and the anomeric effects for chlorine and bromine were estimated to be 2·7 and 3·2 kcal./mole respectively, whereas the value for iodine was believed to be slightly greater than 3·1 kcal./mole.



(178)

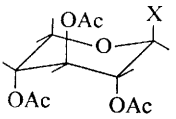
X = Cl, Br, or I



(179)

These values would be expected to decrease appreciably if measured in more polar solvents. Electrostatic calculations gave results in good agreement with those determined experimentally, but the approximations necessarily employed reduce the validity of this approach.[340]

The practical significance of the anomeric effect has been revealed most strikingly by n.m.r. studies of xylopyranosyl halides. Previously it had been shown that the preferred conformations adopted in chloroform solution by the tri-*O*-acetylpyranosyl bromides of β-arabinose, α-lyxose, β-ribose, and α-xylose are those in which the bromine atoms have the axial orientations, so the anomeric effect is sufficiently significant to overcome the steric influence of the axial groups. It has now been shown by n.m.r. spectroscopy, however, that tri-*O*-acetyl-β-D-xylopyranosyl chloride (180) also adopts the chair conformation which allows the halide to be axial despite the fact that this means all the bulky groups on the sugar ring are axial. In the spectrum (100 Mc./sec.) for a chloroform solution all the ring protons except 2-H and 3-H were well resolved, and all showed small vicinal couplings.[339]



(180) X = Cl
(181) X = F

Hall and Manville, using 100 and 220 Mc./sec. techniques, have shown that the fluoro-analogue (181) also adopts this 1*C* conformation and suggested that the tribenzoate does too.[341] The same workers have prepared 3,4,6-tri-*O*-acetyl and -benzoyl-2-deoxy-α-D-*arabino*-hexopyranosyl fluorides as the first nonterminal deoxyglycosyl fluorides and characterised them configurationally by n.m.r. methods.[342] These and other glycosyl fluoride spectra are discussed more fully in the n.m.r. section (see p. 184).

Perhaps rather surprisingly, certain glycosyl fluorides may be characterised with regard to their anomeric configurations by enzymic methods. α-D- And β-D-glucopyranosyl, α- and β-D-galactopyranosyl, α-D-mannopyranosyl, and α-D-xylopyranosyl fluorides were found to be hydrolysed

[341] L. D. Hall and J. F. Manville, *Carbohydrate Res.*, 1967, **4**, 512.
[342] L. D. Hall and J. F. Manville, *Canad. J. Chem.*, 1967, **45**, 1299.

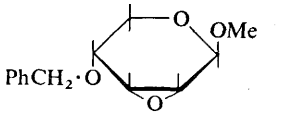
by the appropriate glycosidases at rates similar to those of the corresponding *p*-nitrophenyl glycosides. Since the *manno*-compound was hydrolysed by an enzyme devoid of β-mannosidase activity, the α-configuration can be allocated.[343] A rat intestinal enzyme which hydrolysed α-D-glucopyranosyl fluoride to the free sugar has been subjected to detailed investigation and it was concluded that it is the known α-glucosidase.[344a]

Reductive removal of halogen has been studied in detail (see p. 121) and a new reaction with cadmium dialkyls has been recorded.[213]

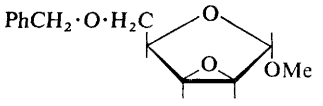
Other Halogenated Derivatives

This topic has been reviewed.[344b]

Fluoro-derivatives with the halogen attached to other than the anomeric position have been studied with increasing activity because of their probable biochemical significance, and several other workers have joined Kent and his colleagues in this area of research. 3-Deoxy-3-fluoro-D-glucose has been synthesised by displacement of the sulphonyloxy-group from 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (104) by the use of tetrabutylammonium fluoride in acetonitrile, followed by removal of the blocking groups.[345] Structural analysis was carried out by conversion of the product to the known 3-deoxy-3-fluoro-D-xylose. A preliminary report has appeared on the metabolism of 3-deoxy-3-fluoro-D-glucose by *Saccharomyces cereviciae*; the compound was oxidised, carbon dioxide was evolved, and the fragments remaining increased the extent of subsequent D-glucose oxidation, consistent with their having an inhibiting effect on polysaccharide synthesis. The substrate activity of the sugar towards various specific metabolic enzymes was assessed and the overall breakdown path was discussed.[346] *N*-Acetyl-3-fluoro-neuraminic acid has been found to inhibit and inactivate *N*-acetyl-neuraminic acid aldolase.[347]



(182)



(183)

Fluorine may also be introduced into carbohydrates by nucleophilic scission of epoxides; treatment of the 2,3-anhydro-derivatives (182) and (183) with potassium hydrogen fluoride in ethane-1,2-diol afforded means for synthesising syrupy 3-deoxy-3-fluoro-D-xylose and crystalline 3-deoxy-3-fluoro-D-arabinose. Several intermediates and derivatives were reported

[343] J. E. G. Barnett, W. T. S. Jarvis, and K. A. Munday, *Biochem. J.*, 1967, **105**, 669.
[344a] J. E. G. Barnett, W. T. S. Jarvis, and K. A. Munday, *Biochem. J.*, 1967, **103**, 699.
[344b] J. E. G. Barnett, *Adv. Carbohydrate Chem.*, 1967, **22**, 177.
[345] A. B. Foster, R. Hems, and J. M. Webber, *Carbohydrate Res.*, 1967, **5**, 292.
[346] R. V. Brunt and N. F. Taylor, *Biochem. J.*, 1967, **105**, 41C.
[347] J. E. G. Barnett, *Biochem. J.*, 1967, **105**, 42P.

and the method was assessed as being generally applicable to the synthesis of fluorohydrins in pure form.[348] 1,3,4-Tri-*O*-benzoyl-2-deoxy-2-fluoro-β-D-ribose and the α- and β-anomers of 1,3,4-tri-*O*-benzoyl-2-deoxy-D-*erythro*-pentose have been subjected to detailed n.m.r. study and all were found to exist in the chair conformation in which the aglycone has the axial orientation.[349] Reactions of 2′-chloro-(and fluoro)-2′-deoxy-β-D-ribofuranosyl nucleosides are mentioned on p. 171.

A new synthesis of chlorodeoxy-sugars involving the use of *NN*-dimethylchloroforminium chloride, which should be generally applicable, has been described by Hanessian and Plessas [185] (Scheme 17). 1,2:3,4-Di-*O*-isopro-

$$ROH \xrightarrow{(Me_2N^+=CHCl)Cl^-} ROCH=\overset{+}{N}Me_2 \xrightarrow{Cl^-} RCl + Me_2N\cdot CHO$$

Scheme 17

pylidene-D-galactopyranose afforded the 6-chloro-6-deoxy-derivative and reaction at secondary sites occurred with inversion. Thus, methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside gave the 3-chloro-3-deoxy-*manno*-derivative, but reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose yielded 6-chloro-6-deoxy-1,2:3,5-di-*O*-isopropylidene-D-glucose; methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-alloside gave 2,3-dichloro-2,3-dideoxy-products of undefined configurations.[185] Reference is made to analogous iodinations on p. 121.

Reaction of methyl β-D-ribopyranoside with sulphuryl chloride and pyridine gave the tri(chlorosulphate), that with chloride ion gave methyl 3,4-dichloro-3,4-dideoxy-α-L-arabinopyranoside 2-chlorosulphate, the stereochemistry of which was established by an alternative synthesis from methyl 2,3-anhydro-β-D-ribopyranoside.[350] Consideration of the conformational factors in the conversion of the triester to the dichloro-compound showed that the transition state for initial displacement at C-4 was less favoured than that for displacement at C-3. The reaction of D-ribose with sulphuryl chloride in pyridine gave β-D-ribopyranosyl chloride 2,3,4-tri(chlorosulphate) shown by n.m.r. spectroscopy to adopt the 1*C* chair conformation. Reaction of chloride ion with methyl 2-*O*-methanesulphonyl-β-D-arabinopyranoside 3,4-di(chlorosulphate) gave the product formed by specific C-4 displacement, namely, methyl 4-chloro-4-deoxy-2-*O*-methanesulphonyl-α-L-xylopyranoside 3-chlorosulphate.[350]

High cystostatic activity has been found for a number of αω-dibromo-αω-dideoxy-polyols (especially the D-mannitol and D-galactitol compounds). This and the corresponding biological properties of the methanesulphonyloxy-analogues have been investigated.[351] The conversion of such compounds to epoxide-containing products has been described.[188, 189]

[348] J. A. Wright and N. F. Taylor, *Carbohydrate Res.*, 1967, **3**, 333.
[349] R. J. Cushley, J. F. Codington, and J. J. Fox, *Carbohydrate Res.*, 1967, **5**, 31.
[350] S. S. Ali, T. J. Mepham, I. M. E. Thiel, E. Buncel, and J. K. N. Jones, *Carbohydrate Res.*, 1967, **5**, 118.
[351] L. Institóris, I. P. Horváth, and E. Csányi, *Arzneim.-Forsch.*, 1967, **17**, 145, 149.

8
Amino-sugars*

Naturally occurring Compounds†

This year several new amino-sugars of novel structure have been isolated from natural products, as well as known ones from new sources.

Gentamicin A and C have each been shown to contain a 3-deoxy-3-methylamino-pentose unit of, as yet, undefined configuration; the one from the latter source, garosamine, also had a *C*-methyl group on C-4.[352] The compound derived from gentamicin A was named gentosamine.[353] Full details have appeared[354] of the structural studies on the antibiotic lincomycin which contains, as a component, a 6-amino-6,8-dideoxyoctose. The antibiotic streptozotocin, a derivative of 2-amino-2-deoxy-D-glucose, has an unusual nitrogen substituent containing an *N*-nitroso-group.[355]

3-Amino-3,6-dideoxy-D-glucose[356, 357] and 4-amino-4,6-dideoxy-derivatives of both D-glucose and D-galactose[358] have been isolated from lipopolysaccharide sources. 3-Amino-3-deoxy-D-glucose has been isolated from the fermentation broth of a *Bacillus* species, now named *Bacillus aminoglucosidus*. The yield was remarkably high (2·6 g./l.). It was claimed that this is the first free amino-sugar to be isolated from a natural source[359, 360] (however, see p. 162).

Large quantities of a compound identified as 2-acetamido-[*N*-(L-aspartamido)]-β-D-glucosylamine have been isolated from the urine of mentally retarded people; this was taken to indicate a fundamental defect

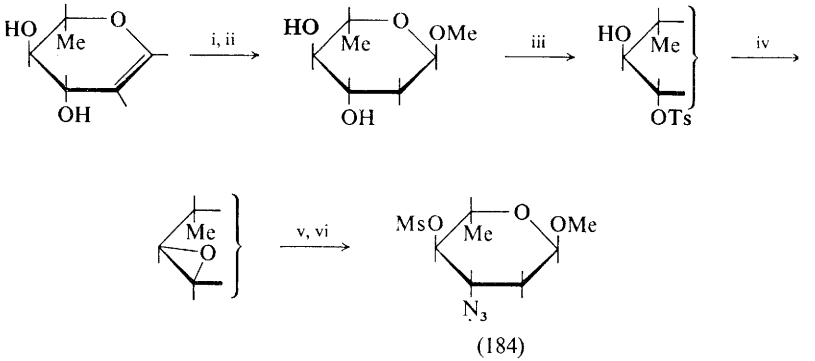
[352] D. J. Cooper and M. D. Yudis, *Chem. Comm.*, 1967, 821.
[353] H. Maehr and C. P. Schaffner, *J. Amer. Chem. Soc.*, 1967, **89**, 6787.
[354] R. R. Herr and G. Slomp, *J. Amer. Chem. Soc.*, 1967, **89**, 2444; W. Schroeder, B. Barrister, and H. Hoeksema, *ibid.*, 2448; G. Slomp and F. A. MacKellar, *ibid.*, 2454; B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *ibid.*, 2459.
[355] R. R. Herr, H. K. Jahnke, and A. D. Argoudelis, *J. Amer. Chem. Soc.*, 1967, **89**, 4808.
[356] R. A. Raff and R. W. Wheat, *J. Biol. Chem.*, 1967, **242**, 4610.
[357] B. Jann, K. Jann, and E. Müller-Seitz, *Nature*, 1967, **215**, 170.
[358] B. Jann and K. Jann, *European J. Biochem.*, 1967, **2**, 26.
[359] S. Umezawa, K. Umino, S. Shibahara, M. Hamada, and S. Omoto, *J. Antibiotics (Tokyo) Ser. A*, 1967, **20**, 355.
[360] S. Umezawa, K. Umino, S. Shibahara, and S. Omoto, *Bull. Chem. Soc. Japan*, 1967, **40**, 2419.

* See also Section 12.
† See Section 20 for further details in appropriate cases.

in glycoprotein metabolism.[361] An unidentified amino-sugar, previously reported in the hydrolysates of polysaccharides containing 2-amino-2-deoxy-D-glucose, is now reported as obtainable from the acid hydrolysis of 2-acetamido-2-deoxy-D-glucose. No evidence, other than that on further treatment it was hydrolysed to the parent amino-sugar, was given.[362a] The disaccharide component of a new nucleotide isolated from hen oviduct has been identified as 2-acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-α-D-glucopyranose.[362b]

Synthesis*

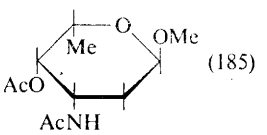
A synthesis[363] of daunosamine, 3-amino-2,3,6-trideoxy-L-*lyxo*-hexose, a component of the antibiotic daunomycin, and one of its D-enantiomorph[364] have been reported. The natural isomer was synthesised from L-rhamnal which was converted[363] into methyl 3-azido-2,3,6-trideoxy-4-*O*-methanesulphonyl-α-L-*arabino*-hexopyranoside (184) by the sequence shown in Scheme 18. This was transformed into methyl 3-acetamido-4-*O*-acetyl-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (methyl diacetyl-daunosaminide)



(184)

Reagents: i, $Hg(OAc)_2$–MeOH; ii, KBH_4; iii, TsCl–pyr; iv, MeOH–MeONa; v, NaN_3; vi, MsCl–pyr.

Scheme 18

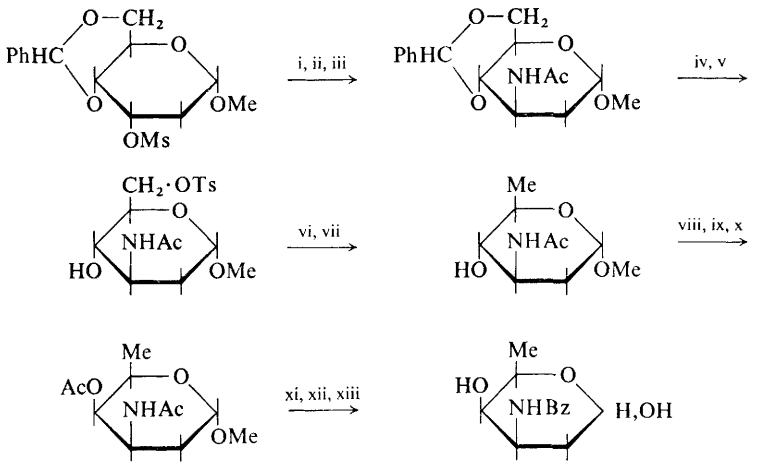


(185)

[361] F. A. Jenner and R. J. Pollitt, *Biochem. J.*, 1967, **103**, 48P.
[362a] D. A. Applegarth, and G. Bozoian, *Nature*, 1967, **215**, 1382;
[362b] Y. Nakanishi, S. Shimixu, N. Takahashi, M. Sugiyama, and S. Suzuki, *J. Biol. Chem.*, 1967, **242**, 967.
[363] J. P. Marsh, jun., C. W. Mosher, E. M. Acton, and L. Goodman, *Chem. Comm.*, 1967, 973.
[364] A. C. Richardson, *Carbohydrate Res.*, 1967, **4**, 422.

* See also Section 6 (pp. 68–72 and 77–80).

(185) by two routes: (i) reaction with sodium benzoate in DMF, mild alkaline hydrolysis, reduction of the azide group, and acetylation; (ii) reduction of the azide group, *N*-acetylation, a Baker solvolysis to give the *cis*-OH,NHAc system and acetylation. The unnatural enantiomorph, isolated as its *N*-benzoyl derivative, was prepared[364] from methyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-methanesulphonyl-α-D-*ribo*-hexopyranoside by the sequence shown in Scheme 19.



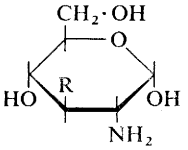
Reagents: i, NaN_3–DMF; ii, H_2–Ni; iii, Ac_2O–MeOH; iv, MeOH–HCl; v, TsCl–pyr; vi, NaI–Me_2CO; vii, H_2–Ni; viii, MsCl–pyr; ix, NaOAc; x, Ac_2O–pyr; xi, 2N-NaOH; xii, Bz_2O–MeOH; xiii, Aq. AcOH.

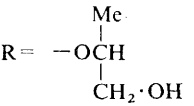
Scheme 19

Two amino-sugars with the *gluco*-configuration have been converted into the corresponding *galacto*-compounds by inversion at C-4. Partial benzoylation of methyl 3-acetamido-3,6-dideoxy-α-L-glucopyranoside gave the 2-*O*-benzoyl derivative, the 4-methanesulphonate of which was solvolysed to give methyl 3-acetamido-3,6-dideoxy-α-L-galactopyranoside.[246] This product was converted into the free sugar, the enantiomorph of that isolated from *Xanthomonas campestris*. These findings confirmed those of an independent study by Czech workers (K. Capek *et al.*, *Coll. Czech. Chem. Comm.*, 1966, **31**, 1854) of the same reactions. Benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-methanesulphonyl-β-D-glucopyranoside, when treated with glacial acetic acid, potassium acetate, and a little water, gave, after deacetylation, benzyl 2-acetamido-2-deoxy-β-D-galactopyranoside.[304]

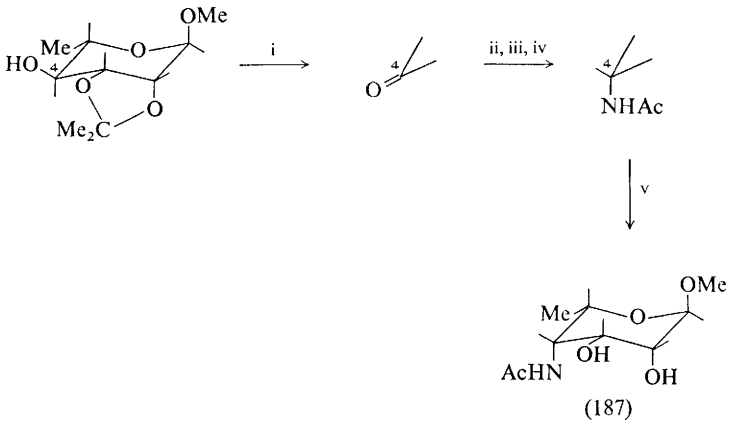
'Reduced muramic acid', 2-amino-2-deoxy-3-*O*-[(*S*)-(hydroxymethyl)-ethyl]-α-D-glucopyranose (186), has been synthesised (as its hydrochloride) by reducing methyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)-ethyl]-α-D-glucopyranoside with lithium borohydride, followed by removal

of the blocking groups.[365] (See p. 107 for the synthesis of muramic acid isomers.) The preparation of a number of derivatives of 2-amino-2-deoxy-D-glucose of potential interest in muramic acid synthesis has been described; these were α- and β-anomers of benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-D-glucopyranoside bearing *N*-benzoyloxycarbonyl, *N*-phenoxycarbonyl, and *N*-chloroacetyl substituents, as well as the *N*-*p*-methoxybenzylidene Schiff bases.[366] In the α-series only, the *N*-bromoacetyl and the *N*-benzoyl derivatives were also prepared.



(186) R = 

The use of aldulose intermediates for the synthesis of amino-sugars has now been extended to 4-amino-4-deoxy-compounds.[367] Methyl 4-acetamido-4,6-dideoxy-L-talopyranoside (187) has been synthesised by the route shown in Scheme 20; the assignment of the *talo*-, rather than the *manno*-configuration, was based on n.m.r. and $[M]_D$ measurements.



(187)

Reagents: i, CrO_3–pyr; ii, NH_2OH; iii, LAH–Et_2O; iv, Ac_2O–EtOH; v, H^+.

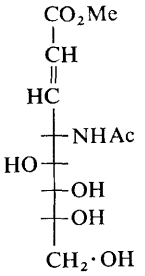
Scheme 20

Two syntheses of 3-amino-3-deoxy-D-glucose derivatives have been reported [302,303] based on displacement of the sulphonyloxy-group by azide ion from, the now readily available, 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-allofuranose (104). In one case,[303] the derived

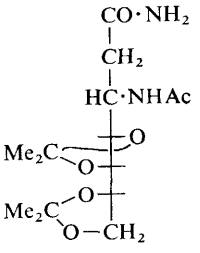
[365] R. W. Jeanloz and E. Walker, *Carbohydrate Res.*, 1967, **4**, 504.
[366] P. H. Gross and R. W. Jeanloz, *J. Org. Chem.*, 1967, **32**, 2759.
[367] S. W. Gunner, W. G. Overend, and N. R. Williams, *Carbohydrate Res.*, 1967, **4**, 498.

3-acetamido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose was converted by standard sequences into 3-amino-3-deoxy-D-xylose.

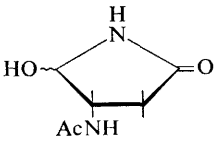
4-Amino-4-deoxy-aldonic acid derivatives have been prepared from the unsaturated compound (188), obtained by treatment of 2-acetamido-2-deoxy-D-glucose with a Wittig reagent.[368] Addition of ammonia to the



(188)



(189)



(190)

double bonds of the products from Wittig reactions on aldoses has given 3-amino-3-deoxy-derivatives, such as (189) which on hydrolysis and periodate oxidation gave the lactam (190).[369]

1-Amino-1-deoxyheptitols having the D-*glycero*-D-*gulo*-, the D-*glycero*-D-*galacto*-, and the D-*glycero*-D-*manno*-configuration have been described. The first was made by reduction of the appropriate oxime, the other two from reduction of the corresponding 1-deoxy-1-nitro-compounds.[370] Hydrogenation of a D-glucose–methylamine mixture over Raney nickel gave 1-deoxy-1-methylamino-D-glucitol in good yield.[371]

Derivatives of 5-amino-5-deoxy-D-ribofuranose and -xylofuranose have been described.[310] Selective displacement of the primary sulphonyloxy-group in methyl 2,3,5-tri-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside by azide ion gave the 5-azido-compound, which was transformed into the 5-acetamido-derivative (159). The neighbouring-group effect of the acetamido-group permitted displacement of the C-3-sulphonyloxy-group to give the xylofuranose derivative (160).

The synthesis of 1-, 4-, and 5-amino-ketose derivatives has been described. Selective displacement of the 5-sulphonyloxy-group from methyl 1,3-*O*-benzylidene-4,5-di-*O*-methanesulphonyl-α-L-sorbopyranoside (124) and from the corresponding β-D-fructopyranoside derivative (126) with azide ion has been described.[312] Reduction of the products gave the appropriate 5-amino-5-deoxy-compounds (D-*fructo*- and L-*sorbo*- respectively).

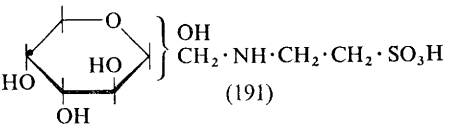
[368] B. A. Dmitriev and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2483.

[369] B. A. Dmitriev, N. E. Bairamova, A. A. Kost, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2491.

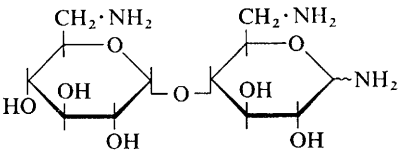
[370] H. J. F. Angus and N. K. Richtmyer, *Carbohydrate Res.*, 1967, **4**, 7.

[371] V. Ya. Yakovleva, A. D. Gorbunova, G. I. Vishnevskaya, S. S. Kirienko, D. F. Yavorskii, and L. M. Yagupol'skii, *Khim. Farm. Zhur.*, 1967, **1**, 51.

Continuing their studies on the nitromethane cyclisation (see also p. 107), Litchenthaler and his group have cyclised the dialdehyde obtained from oxidation of benzyl β-D-fructopyranoside with nitromethane to give, after reduction, derivatives of 4-amino-4-deoxy-α-L-sorbopyranoside and -β-D-tagatopyranoside.[372] The 1-amino-1-deoxy-fructose derivative (191) has been synthesised from the corresponding 4,6-*O*-benzylidene-glucosylamine *via* an Amadori rearrangement.[373]



(191)



(192)

The synthesis of some amino-sugar disaccharides has been mentioned already in this Report.[83–86, 374] 6,6′-Diamino-6,6′-dideoxymaltosylamine (192) and 6,6′-diamino-6,6′-dideoxytrehalose have been prepared as antibiotic analogues.[375a] The first was prepared by preferential toluene-*p*-sulphonylation of maltose, followed by acetylation, bromination, and reaction with azide ion, then deacetylation and reduction. The trehalose compound was prepared by a similar route.

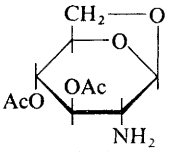
Enzymes from *Saccharomyces carlsbergensis* yeast catalysed the synthesis of 2-acetamido-2-deoxy-6-*O*-(α-D-galactopyranosyl)-D-glucose (*N*-acetylmelibiosamine) from D-galactose or phenyl α-D-galactopyranoside and 2-acetamido-2-deoxy-D-glucose.[375b] A second disaccharide, tentatively identified as 2-acetamido-2-deoxy-3-*O*-(α-D-galactopyranosyl)-D-glucose, has been isolated from the reaction with D-galactose and 2-acetamido-2-deoxy-D-glucose as substrates.

Reactions

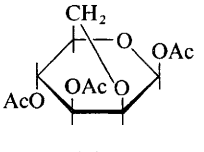
Several papers have appeared on the nitrous acid deamination of amino-sugars, one of them using the reaction as the key step in the synthesis of a branched-chain nucleoside (see p. 135). Deamination of D-glucosamine diethyl dithioacetal hydrochloride gave 2,5-anhydro-D-glucose diethyl

[372] F. W. Lichtenthaler and H. K. Yahya, *Chem. Ber.*, 1967, **100**, 2389.
[373] K. Heyns, H. Behre, and H. Paulsen, *Carbohydrate Res.*, 1967, **5**, 225.
[374] K. Heyns, R. Harrison, and H. Paulsen, *Chem. Ber.*, 1967, **100**, 271.
[375a] S. Umezawa, T. Tsuchiya, S. Nakada, and K. Tatsuta, *Bull. Chem. Soc. Japan*, 1967, **40**, 395;
[375b] M. J. Clancy and W. J. Whelan, *Arch. Biochem. Biophys.*, 1967, **118**, 730.

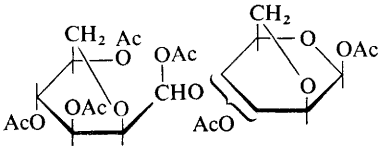
dithioacetal as the major product which was converted by standard sequences into 2,5-anhydro-D-glucose.[376] Treatment of 2-amino-1,6-anhydro-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranose (193) with nitrous acid in acetic acid gave 1,3,4-tri-*O*-acetyl-2,6-anhydro-D-mannose (194); acid



(193)



(194)



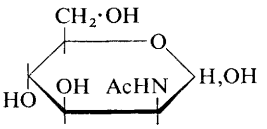
(195)

hydrolysis gave the unblocked D-mannose derivative, which was reduced to 2,6-anhydro-D-mannitol.[377] Acetylation of 2,6-anhydro-D-mannose with acetic anhydride in pyridine gave a hexa-*O*-acetyl disaccharide, also obtained by deamination of 2-amino-1,6-anhydro-2-deoxy-β-D-glucose hydrochloride, followed by acetylation. The partial structure (195) was assigned to this product.[377] Deamination of 1-amino-1-deoxy-D-*glycero*-D-*galacto*-heptitol gave perseitol (by replacement of the amino-group by an hydroxy-group), together with two other products believed to be anhydro-heptitols.[370]

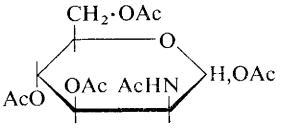
A number of papers have appeared from Fletcher's school on the acetylation of amino-sugars, particularly with isopropenyl acetate and toluene-*p*-sulphonic acid. This study, begun in 1966 with 2-acetamido-2-deoxy-D-glucose, has now been extended to other sugars. 2-Acetamido-2-deoxy-D-mannose (196) reacted with isopropenyl acetate and toluene-*p*-sulphonic acid in a different manner to the *gluco*-isomer to give a mixture of at least six products.[378] The main products (31%) were the anomeric 1,3,4,6-tetra-*O*-acetyl derivatives (197), together with compounds assigned the following structures: 2-(D-*glycero*-1,2-diacetoxyethyl)-4-(*N*-acetylacetamido)-furan (198) (13%), 3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (199) (14%), 1,1,3,4,5,6-hexa-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-D-mannose (200) (0·36%), and 4-acetamido-2-(D-*glycero*-1,2-diacetoxyethyl)furan (201) (3%). These products were characterised by n.m.r. spectroscopy and chemical methods. The

[376] J. Defaye, *Bull. Soc. chim. France*, 1967, 1101.
[377] F. Micheel, W. Neier, and T. Riedel, *Chem. Ber.*, 1967, **100**, 2401.
[378] N. Pravdić and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 1806.

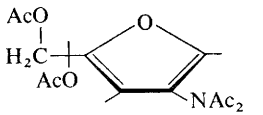
mechanism for the production of the unsaturated sugar (199) was considered[379] to involve intermediate formation of the per-*O*-acetyl-*N*-acetylacetamido-α-anomer with diaxially orientated groups at C-1 and C-2, from which elimination then occurred, with participation of one of the *N*-acetyl groups. The corresponding peracetyl-β-anomer did not undergo similar elimination. α-Compounds which also gave the glycal derivative



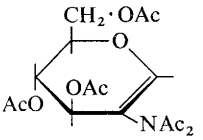
(196)



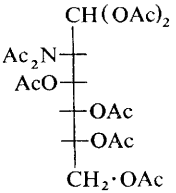
(197)



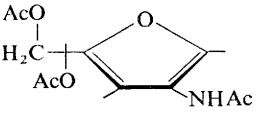
(198)



(199)



(200)



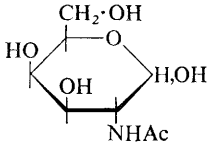
(201)

(199) with isopropenyl acetate were the corresponding ethyl thioglycoside and 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-mannopyranose. It was further suggested[379] that the furan derivative (198) arose by a similar elimination from 1,3,5,6-tetra-*O*-acetyl-2-(*N*-acetylacetamido)-2-deoxy-α-D-mannofuranose. Reaction of 2-acetamido-2-deoxy-D-galactose (202) with isopropenyl acetate in a similar fashion has also been shown[380] to give several products of the same type as those formed from the *manno*-isomer; the compounds isolated (with yields) were (203) to (207). Compound (205) was shown to be the precursor of the unsaturated sugar (207) and it is the Reviewers' opinion that the latter may have arisen by a 1,2-elimination followed by an allylic rearrangement.

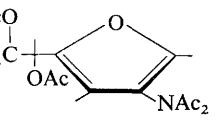
Acetylation of 2-acetamido-2-deoxy-D-mannose (196) using acetic anhydride and limited amounts of zinc chloride gave the expected anomeric 1,3,4,6-tetra-*O*-acetyl derivatives (197). However, when a high proportion of catalyst and a longer reaction time were used, the oxazoline (208) was also produced;[381] this was readily hydrolysed to 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-mannopyranose (which may account for the occurrence of this compound in the initial reaction products) or converted into the methyl or benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-mannopyranosides. Similar oxazolines were shown[381] to be produced by zinc chloride-catalysed acetylation of 2-acylamido-2-deoxy-derivatives of D-glucose and

[379] N. Pravdić and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 1811.
[380] N. Pravdić and H. G. Fletcher, jun., *Croat. Chem. Acta*, 1967, **39**, 71.
[381] N. Pravdić, T. D. Inch, and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 1815.

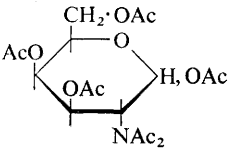
of D-galactose. The oxazolines where thought to be formed by the Lewis acid-catalysed intramolecular displacement of a *trans*-acetoxy-group on C-1.



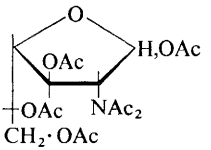
(202)



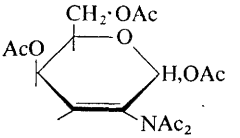
(203) (7·8%)



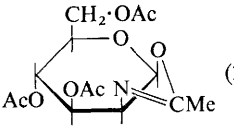
(204) α-(17%)
(205) β-(0·3%)



(206) (22%)



(207) (4·5%)

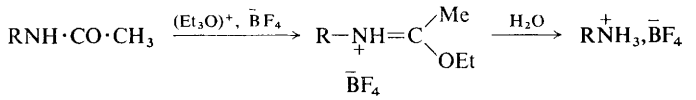


(208)

Following the successful syntheses of perbenzyl derivatives of 2-acetamido-2-deoxy-D-glucose and -galactose with benzyl bromide, barium oxide, and barium hydroxide octahydrate in DMF, the same reaction was attempted on 2-acetamido-2-deoxy-D-mannose (196). However, appreciable amounts of the β-*gluco*-product were formed [382] showing that epimerisation at C-2 preceded benzylation. Consequently, (196) was treated with benzyl alcohol in the presence of boron trifluoride and the products were then acetylated. The main component of the product mixture was tentatively identified as benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside, another as the β-anomer, and a third as benzyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-α-D-mannofuranoside.[382]

A new method for the removal of *N*-acetyl groups from acetamido-sugars has been described in a preliminary report.[383] The treatment (Scheme 21) involved use of triethyloxonium fluoroborate in an inert solvent, such as dichloromethane; the intermediate *O*-ethylacetamidium fluoroborate was readily decomposed by water to the parent amino-sugar salt. The reaction could be carried out in the presence of esters, acetals, and glycosidic links.

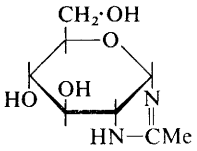
[382] J. R. Plimmer, N. Pravdić, and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 1982.
[383] S. Hanessian, *Tetrahedron Letters*, 1967, 1549.



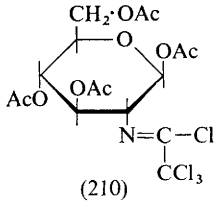
Scheme 21

Use of the diphenoxyphosphinyl group [—$PO(OPh)_2$] as a non-participating, amino-protecting group has been described. One use was in the synthesis of the amino-sugar disaccharides:[83, 374] the other was in the synthesis of nucleosides of 2-amino-2-deoxy-D-glucose.[384] The trichloro- and trifluoro-acetyl protecting groups have also been employed in such syntheses.[385]

A brief report has described the reaction of 2-amino-2-deoxy-D-glucose with ethyl iminoacetate hydrochloride [$CH_3C(OEt)$=NH,HCl] in DMF which gave the 2-acetamido-2-deoxy-derivative (presumably after hydrolysis) and, as the main product (30% after chromatography), 2-methyl-D-glucopyranosyl-[1′,2′:4,5]-2-imidazoline (209), characterised spectroscopically.[386] The reaction of phosphorous pentachloride with 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose gave 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-tetrachloroethylideneamino-β-D-glucopyranose (210),



(209)



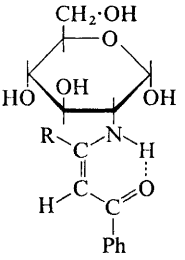
(210)

characterised spectroscopically and by hydrogenolysis to the corresponding 2-amino-2-deoxy-derivative.[387] It was shown that phosphorous pentachloride did not give (210) when the corresponding 2-trichloroacetamido-2-deoxy-derivative was used, and so it was proposed that the unique chlorine was the first to enter to give the group —N=C(Cl)CH_3, which was then further chlorinated.

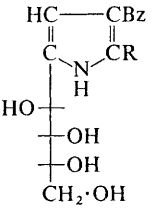
Methyl 2-acetamido-2,6-dideoxy-α-D-altroside has been prepared by conventional sequences from methyl 2-acetamido-2-deoxy-α-D-altroside.[388] This was a hitherto unknown compound of this class; the *gulo*- and *ido*-isomers are still unknown.

[384] M. L. Wolfrom, P. J. Conigliaro, and E. J. Soltes, *J. Org. Chem.*, 1967, **32**, 653.
[385] M. L. Wolfrom and H. B. Bhat, *J. Org. Chem.*, 1967, **32**, 1821.
[386] M. H. Fischer and B. A. Lewis, *Chem. and Ind.*, 1967, 192.
[387] C. H. Bolton, A. M. Dempsey, and L. Hough, *Chem. Comm.*, 1967, 658,
[388] A. Zobáčová, V. Heřmánková, and J. Jarý, *Coll. Czech. Chem., Comm.*, 1967, **32**, 3560.

As part of the study of the reactions of sugars with β-dicarbonyl compounds, the reaction of 2-amino-2-deoxy-D-glucose with benzoylacetaldehyde and with 1-phenylbutan-1,3-dione has been investigated.[389] The products, characterised by spectroscopic studies of their *O*-acetyl derivatives, were respectively, 2-deoxy-2-[1-(3-oxo-3-phenyl-1-propenyl)amino]-D-glucose (211) and 2-deoxy-2-[2-(4-oxo-4-phenyl-2-butenyl)amino]-D-



(211) R = H
(212) R = Me



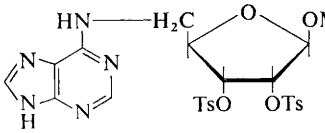
(213) R = H
(214) R = Me

glucose (212). Cyclisation of these products gave the pyrrole derivatives (213) and (214), the polyhydroxy-side-chains of which could be oxidised to an aldehyde group by periodate.

A number of *N*-salicylidene derivatives of amino-sugars have been prepared and a study has been made of their tautomerism[390] and o.r.d. spectra.[391] The results of the latter study have been applied to several amino-sugar-containing antibiotics.[392]

1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride has been condensed with *N*-benzoyloxycarbonyl-L-amino-acids in the presence of DCC, followed by removal of the *N*-blocking group by hydrogenolysis.[393] The products were shown to be much less stable than their known β-analogues.

A 'reverse nucleoside' (215) has been synthesised from a methyl 5-amino-5-deoxy-β-D-ribofuranoside derivative.[310] The following glycoside derivatives

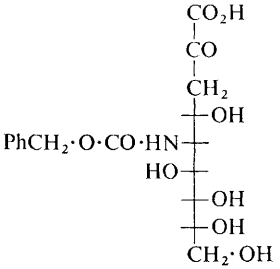


(215)

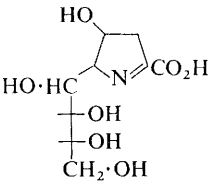
[389] A. Gómez-Sánchez, M. Gómez Guillén, and U. Scheidegger, *Carbohydrate Res.*, 1967, **3**, 486.
[390] S. Inouye, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1540.
[391] S. Inouye, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1557.
[392] S. Inouye, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1609.
[393] M. Liefländer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1967, **348**, 477.

of 2-acetamido-D-glucose have been described: 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-4-hydroxy-L-proline methyl ester (19)[110] and 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-DL-threonine methyl ester (18).[109] Studies of the hydrolysis of *O*-seryl and *O*-threonyl[108] and of *o*- and *p*-nitrophenyl[104] glycosides of 2-acetamido-2-deoxy-D-glucose have been reported.

Hydrogenolysis of *N*-benzyloxycarbonyl-neuraminic acid (216) did not give the expected unblocked amino-product, but instead its internal Schiff's base, 4-hydroxy-5-(D-*gluco*-1,2,3,4-tetrahydroxybutyl-1-pyrroline-2-carboxylic acid (217). The equilibrium between the free acid and (217)



(216)

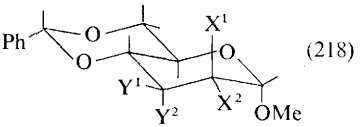


(217)

was shown to be pH-dependent, the former only occurring in strongly acidic solutions.[394] Anomeric phenyl glycosides of neuramic acid have been described.[65a, b] For use in enzymic studies the *N*-propionyl, *N*-butyryl, and *N*-benzoyl-neuraminic acids have been prepared, and converted into their benzyl glycosides; anomeric methyl glycosides were made of the *N*-benzoyl compound only.[395]

Physical Measurements

A study has been made of the cuprammonium complexing of the eight amino-sugar derivatives represented by system (218), by both optical rotational shifts and by conductance changes;[396] the results paralleled those of Reeves for the corresponding diols. This technique should be



(218)

If an X = OH, then a Y = NH_2 or *vice versa*: the rest are H.

[394] W. Gielen *Hoppe-Seyler's Z. Physiol. Chem.*, 1967, **348**, 329.
[395] P. Meindl and H. Tuppy, *Monatsh.*, 1967, **97**, 1628.
[396] C. B. Barlow and R. D. Guthrie, *J. Chem. Soc.* (*C*), 1967, 1194.

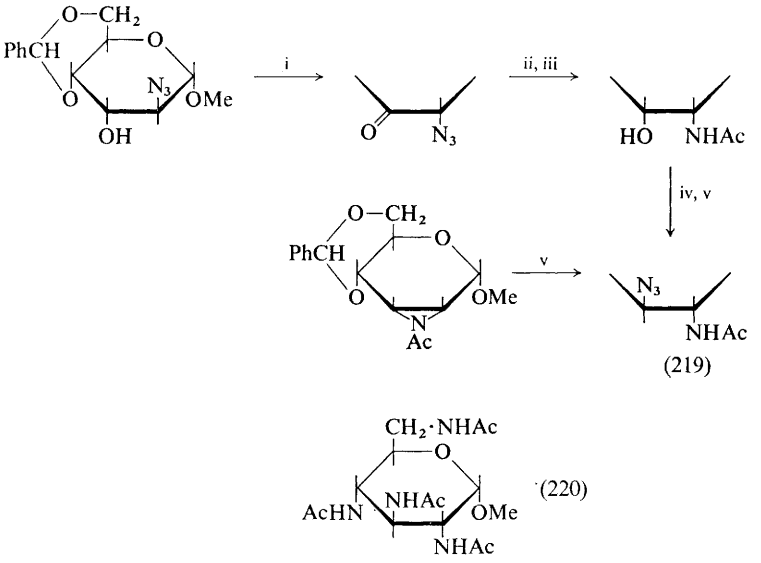
of use in determining the structures of unknown amino-sugars. The anomalous optical rotations of the anomeric 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-D-glucopyranoses have been investigated (see p. 190).

A detailed study has been made of the n.m.r. spectra of acetylated amino-sugars, with particular reference to the specific assignment of the acetyl methyl signals (see p. 182).

Diamino- and Polyamino-sugars

Reduction of the diphenylazo-mannoside resulting from the condensation of periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucoside and phenylhydrazine in DMF followed by acetylation gave methyl 2,3-diacetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannoside, probably the best route yet available to this diaminohexose.[397] A new synthesis of 2,3-diamino-2,3-dideoxy-D-glucose derivatives has been described, based on the reaction of ammonia with methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside or the corresponding nitro-olefin [398a] (cf. p. 107), followed by reduction of the nitro-group.

Methyl 2-acetamido-2-deoxy-α-D-altroside has been converted by standard reactions *via* the 6-*O*-toluene-*p*-sulphonyl derivative into the



Reagents: i, DMSO–Ac_2O; ii, $NaBH_4$; iii, Ac_2O–EtOH; iv, MsCl–pyr; v, NaN_3–DMF.

Scheme 22

[397] B. E. Davison, R. D. Guthrie, and D. Murphy, *Carbohydrate Res.*, 1967, **5**, 449.
[398a] H. H. Baer and T. Neilson, *J. Org. Chem.*, 1967, **32**, 1068.

corresponding 2,6-diacetamido-2,6-dideoxy derivative.[388] This leaves only one 2,6-diamino-2,6-dideoxy-hexose as yet unsynthesised, namely the *talo*-isomer.

1′,6,6′-Triamino-1′,6,6′-trideoxy-sucrose has been prepared from sucrose by conventional sequences, *via* the tri-*O*-toluene-*p*-sulphonyl derivative.[375a]

Reaction of nitromethane with simple dialdehydes in the presence of primary amines has been shown to give cyclic nitrodiamines, that may be reduced to triamino-derivatives.[398b] Extension of the reaction to the dialdehyde from the periodate oxidation of 1,6-anhydro-β-D-glucopyranose using benzylamine gave 1,6-anhydro-2,4-bis(benzylamino)-2,3,4-trideoxy-3-nitro-β-D-idose (53%). Reduction and hydrogenolysis gave the expected triamino-trideoxy-compound, the *N*-acetyl derivative of which was used for stereochemical characterisation by n.m.r. methods.[398b]

A preliminary report has described the first syntheses of derivatives of 2,3,4,6-tetra-amino-2,3,4,6-tetradeoxyhexoses.[305] The key intermediate in the preparation of methyl 2,3,4,6-tetra-acetamido-2,3,4,6-tetradeoxy-α-D-glucoside (220) was methyl 2-acetamido-3-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucoside (219) which was prepared by the two routes shown in Scheme 22. This was converted into the diacetamido-dideoxy-glucoside which was then transformed into (220) by standard reactions. Methyl tetra-acetamido-α-D-idopyranoside was prepared in a similar way *via* the readily accessible methyl 3-acetamido-2-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-altroside.

[398b] F. W. Lichtenthaler, T. Nakagawa, and A. El-Scherbiney, *Angew. Chem. Internat. Edn.*, 1967, **6**, 568.

9
Hydrazones, Osazones and Formazans

The mutarotation of 3,4,5,6-tetra-*O*-benzoyl-D-glucose phenylhydrazone, previously assigned by Micheel and Dijong (*Chem. Ber.*, 1964, **97**, 2409) to a phenylhydrazone ⇌phenylazo tautomerism, has been shown to be due to formation of a phenylazo-hydroperoxide.[399] The phenylazo-hydroperoxide derived[400] from oxygenation of penta-*O*-acetyl-D-galactose phenylhydrazone gave a 60% yield of D-lyxose after treatment with dilute methanolic sodium methoxide; reaction with zinc and acetic acid gave the original hydrazone, and with sodium iodide in acetic acid, the *N*-phenyl-galactonic acid hydrazide. By a similar degradation, L-rhamnose phenylhydrazone was converted into 5-deoxy-L-arabinose. It was also mentioned in this brief report[400] that derivatives of D-glucose and D-xylose had been similarly treated.

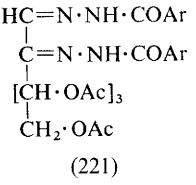
A study of the i.r. spectra of the phenylhydrazones of D-galactose, D-glucose, and D-mannose in the solid state showed that they have cyclic structures, contrary to conclusions based on acetylation experiments and the formation of formazans.[401] Known cyclic phenylhydrazones (*X*-ray method) have been shown to react in the acylic form in the above reactions, suggesting that they are not suitable for differentiating between acyclic and cylic forms.

Rates have been reported for the reaction of *p*-substituted phenylhydrazines and eight common monosaccharides. The course of the reaction was not characterised in every case but it was found that rates increased in going from ketohexoses to aldohexoses to aldopentoses, and from systems with conformationally stable rings to those less stable.[402] Alternatively, rates decreased with increasing electron-withdrawing power of the aryl substituents.

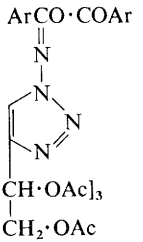
El Khadem and his group continue to report on the chemistry of hydrazones and related compounds. Two papers have appeared on the synthesis of bis(acylhydrazones) of D-*arabino*-hexulose:[403, 404] it was shown

[399] J. Buckingham and R. D. Guthrie, *J. Chem. Soc.* (*C*), 1967, 2268.
[400] M. Schulz and L. Somogyi, *Angew. Chem. Internat. Edn.*, 1967, **6**, 168.
[401] H. S. Blair and G. A. F. Roberts, *J. Chem. Soc.* (*C*), 1967, 2425.
[402] H.-H. Stroh and P. Golüke, *Z. Chem.*, 1967, **7**, 60.
[403] H. El Khadem, G. H. Labib, and M. A. Nashed, *Carbohydrate Res.*, 1967, **3**, 509.
[404] H. El Khadem and M. A. E. Shaban, *Carbohydrate Res.*, 1967, **4**, 416.

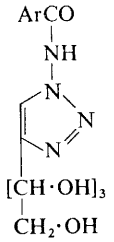
that their formation was catalysed by aromatic amines. Bis(aroylhydrazones) (221) have also been prepared from the ulose tetra-acetate with aroylhydrazines.[405] Deacetylation gave the parent hydroxy-compounds and not anhydro-osazones, as do arylosazones. Oxidation of the products



(221)



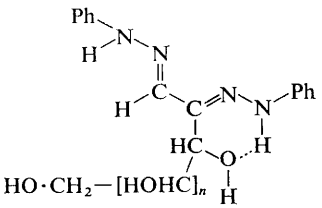
(222)



(223)

with iodine and mercuric oxide gave 1-α-aroyloxyarylideneamino-1,2,3-triazoles (222), which were hydrolysed to the compounds (223). Bis(semicarbazones),[403] 2-acylhydrazone-1-phenylhydrazones, and 2-acylhydrazone-1-(2-methyl-2-phenylhydrazones)[404] of D-*arabino*-hexulose have also been described.

Investigation by n.m.r. and u.v. spectroscopy and the formazan reaction of the final form of sugar osazones after mutarotation has shown it to be the N—H · · · O chelated structure (224).[406]

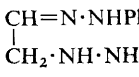


(224)

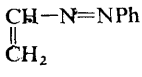
The mechanism of osazone formation continues to be studied. Simon and his group have studied a postulated but hitherto uninvestigated intermediate, in its simplest form a derivative of glycolaldehyde (225).[407] An intermolecular oxidation–reduction step was most probably excluded, the key reaction being an intramolecular oxidation–reduction with aniline elimination. Compound (225) also underwent elimination of phenylhydrazine to give the azo-ene (226). The rapid formation of the osazone

[405] H. El Khadem and M. A. E. Shaban, *J. Chem. Soc.* (*C*), 1967, 519.
[406] L. Mester, E. Moczar, G. Vass, and A. Schimpl, *Carbohydrate Res.*, 1967, **5**, 406; *Tetrahedron Letters*, 1967, 2943.
[407] H. Simon, G. Heubach, and H. Wacker, *Chem. Ber.*, 1967, **100**, 3106.

from (225) that occurred in anhydrous acetic acid in the absence of phenylhydrazine was assumed to occur *via* (226). Reaction of D-glucose or D-mannose phenylhydrazones with acetic anhydride in pyridine gave the known 3,4,5,6-tetra-*O*-acetyl-D-*arabino*-hex-1-en-3,4,5,6-tetrol which, with phenylhydrazine, gave the osazone. The rate of the formation of the azo-ene from the *gluco*-phenylhydrazone was ten times faster than from the *manno*-isomer suggesting that it is this reaction which reflects the differences in rates of osazone formation from different sugars.



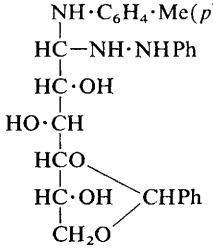
(225)



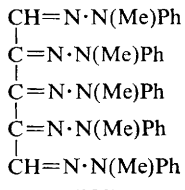
(226)

Simon's group have also shown that dilute alcoholic potassium hydroxide causes rapid hydrogen exchange at C-1 in glucosazone and also degradation to glyoxal bisphenylhydrazone, 2,4-dihydroxybutyric acid, and a small amount of 3,6-anhydro-D-*ribo*-hexulose phenylosazone.[408] D-Glucose and D-mannose phenylhydrazones, besides showing hydrogen exchange at C-1, were degraded to aniline and *N*-phenylpyrazole.

Micheel and his co-workers[409] have shown that the 1-deoxy-1,1-*N*-bis(acetal) of 4,6-*O*-benzylidene-D-glucose (227) was converted by oxalic acid and phenylhydrazine in dioxan into 3-deoxy-D-*erythro*-hexulose bisphenylhydrazone.



(227)



(228)

The Feisers' hypothesis that chelation is responsible for limiting the reaction of sugars with phenylhydrazine to the osazone stage has been examined using *N*-methyl-*N*-phenylhydrazine[410] which would preclude the type of hydrogen-bond invoked. Reaction of this arylhydrazine with a variety of polyhydroxycarbonyl compounds containing from three to six

[408] H. Simon and W. Moldenhauer, *Chem. Ber.*, 1967, **100**, 3121.
[409] F. Micheel, S. Degener, and I. Dijong, *Annalen*, 1967, **701**, 233.
[410] O. L. Chapman, W. J. Welstead, T. J. Murphy, and R. W. King, *J. Amer. Chem. Soc.*, 1967, **89**, 7005.

carbon atoms gave products bearing an *N*-methyl-*N*-phenylhydrazone group at each carbon; for example, (228) was produced from a pentose. The name 'alkazone' was suggested for the products, and the geometrical isomerism exhibited by them was discussed. These results, although consistent with the chelation theory, do not prove it.

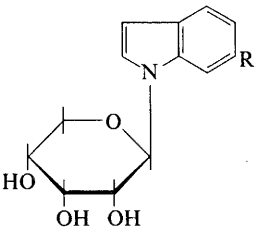
Formazans of free aldoses have been prepared from 2-hydrazinobenzimidazoles.[411]

[411] Yu. A. Sedov, N. P. Bednyagina, and I. Ya. Postovskii, *Zhur. obshchei Khim.*, 1967, **37**, 139.

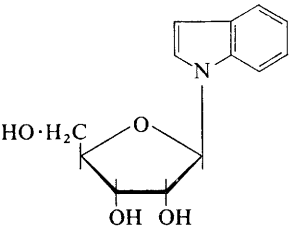
10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines

Condensation of 3- or 6-aminoflavones with D-glucose, D-galactose, D-mannose, D-ribose, L-arabinose, or L-rhamnose gave crystalline glycosylamines, which were assigned β-configurations on the basis of their optical rotations.[412] The synthesis of the D-ribopyranosylindole derivatives (229)



(229) R = NO_2 or NH_2



(230)

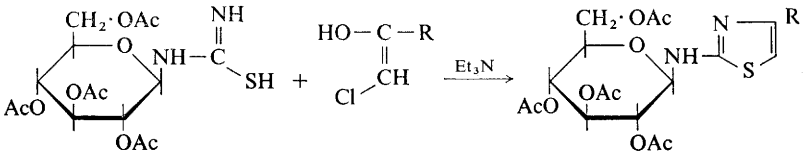
has been reported.[413] Condensation of 5-*O*-trityl-D-ribose with indoline followed by acetylation, dehydrogenation, and removal of the blocking groups gave 1-(β-D-ribofuranosyl)indole (230), a nucleoside model.[414]

A number of 2-acetamido-1-*N*-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylamines and 2-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylacetamides have been prepared as model compounds for glycoprotein studies.[415]

Several aryl-substituted *N*-(β-D-glucopyranosyl)-benzamides have been synthesised from 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine and the appropriate aroyl chloride; thiobenzamide derivatives were prepared by treatment of the products with phosphorous pentasulphide. The effect of the aryl substituent on the rate of acid hydrolysis of the products was investigated.[416]

[412] J. Sykulski, *Roczniki Chem.*, 1967, **41**, 675, 1059.
[413] M. N. Preobrazhenskaya, L. A. Savelyeva, and N. N. Suvorov, *Khim. geterotsikl. Soedinenii*, 1967, 692.
[414] M. N. Preobrazhenskaya, M. M. Vigdorchik, and N. N. Suvorov, *Tetrahedron*, 1967, **23**, 4653.
[415] J. Yoshimura, H. Hashimoto, and H. Ando, *Carbohydrate Res.*, 1967, **5**, 82.
[416] R. Schmidt and G. Wagner, *Pharmazie*, 1967, **22**, 551.

The synthesis of ribo-furanosides and -pyranosides of 2-hydroxypyridine has been reported and their rearrangement to *N*-glycosides of 2-pyridone investigated.[91] A number of new glycosylamines have been synthesised by conversion of a simple aglycone into a more complex one, as shown in the example [(231) to (232)].[417] TMS derivatives of *N*-arylglucosylamines, useful for their separation, have been described.[418]



(231) (232)

Mutarotation of 2,3,4,6-tetra-*O*-acetyl-*N*-*p*-chlorophenyl-D-glucopyranosylamine has been studied in the presence of substituted benzoic acids. Linear plots were obtained for first-order reactions and for log K *vs.* pK_a and the Hammett constants.[419] The i.r. spectra of a wide selection of *N*-acyl-glycosylamines and 1,1-bis(acylamido)-1-deoxyalditols and their esters have been recorded and discussed in detail with particular emphasis on structural correlation.[420]

Glycosylurea Derivatives

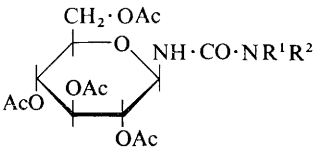
D-Glucosyl-, and D-galactosyl-, D-xylosyl-, and D-arabinosyl-urea have been prepared under controlled conditions; physical and chromatographic properties of the anomers were given, and a kinetic study of the acid hydrolysis of the *gluco*-product was carried out.[421] The synthesis of a large number of new substituted tetra-*O*-acetyl-D-glucopyranosyl thiosemicarbazones has been reported.[417] A number of *N'*-substituted glycosylurea derivatives have been synthesised, for example (233)[422] and (234).[423]

Azides

Sugar azides continue to be popular intermediates for the synthesis of amino-sugars (see Section 8). A smooth reduction of the azido-group to an amino-group in good yield has been reported with sodium borohydride in methanolic DMF.[424]

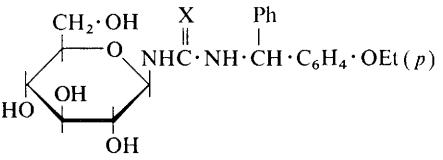
[417] R. Bognar, L. Somogyi, L. Szilagyi, and Z. Györgydeak, *Carbohydrate Res.*, 1967, **5**, 320.
[418] R. E. Kadunce, *J. Chromatog.*, 1967, **30**, 204.
[419] T. Jasinski and K. Smiataczowa, *Roczniki Chem.*, 1967, **41**, 579.
[420] R. S. Tipson, A. S. Cerezo, V. Deulofeu, and A. Cohen, *J. Res. Nat. Bur. Stand., Sect. A*, 1967, **71**, 53.
[421] N. G. Shkantova, M. S. Dudkin, and S. I. Grinshpun, *Zhur. priklad. Khim.*, 1967, **40**, 164.
[422] H. Dorn and M. Schütt, *Z. Chem.*, 1967, **7**, 182, 276.
[423] H. Dorn and H. Welfle, *Pharmazie*, 1967, **22**, 558.
[424] Y. Ali and A. C. Richardson, *Carbohydrate Res.*, 1967, **5**, 441.

The reactions of several azido-sugars with anhydrous hydrazine have been studied.[425] The reaction was remarkably sensitive to the configuration of the azide group and to the nature of the neighbouring group. The results can be summarised as follows: *vic*-azido-alcohols gave the corresponding amino-alcohol; *trans*-(*ax*, *ax*) and *cis*-(*ax*, *eq*)-azido-sulphonates



(233)

$R^1 = R^2 = -CH_2-$
$R^1 = R^2 = CH_2 \cdot CH_2Cl$
$R^1 = H$, $R^2 = N(CH_2 \cdot CH_2Cl)_2$



(234)

X = O or S

gave the corresponding olefinic sugar; *trans*-(*eq*, *eq*)-azido-sulphonates gave deoxy-sulphonates in which the HCN_3 group had been reduced to methylene, the sulphonyl group being unaffected. No mechanism was proposed for these reactions.

Nitro-compounds

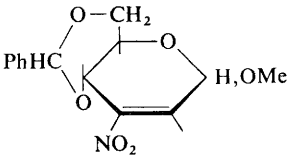
Additions to the nitro-olefin (235), prepared from methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucoside (237), either prior to the reaction or *in situ*, have been studied with many reagents.[398a, 426, 427] Substituents introduced in high yield were methoxy-, ethoxy-, benzyloxy-, benzylthio-, diethylamino-, piperidino-, and *N*(ethoxycarbonylmethyl)-amino-, all with the 3-nitro-2-X-*gluco*-configuration.[426] Addition of ammonia [398a] gave the 2-amino-2,3-dideoxy-3-nitro-*gluco*-compound which was converted into several derivatives of 2,3-diamino-2,3-dideoxy-D-glucose. A second product, not yet characterised, was also isolated from the mixture obtained by addition of ammonia to (235). Addition of isopropyl L-lactate or its D-isomer to the nitro-olefin (235) gave the *gluco*-diastereoisomers (239) in high yield.[427] Addition of racemic lactate gave the DL and DD ethers in a ratio of 10 : 1. By standard procedures these products were converted into the isomers (240) of muramic acid.

Attempts have now been made to extend these nitro-olefin studies into the α-series.[428] Acetylation of methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-α-D-glucopyranoside gave the expected acetate (238) which, on treatment with sodium hydrogen carbonate, lost acetic acid to give the nitro-olefin (236) which on hydrogenation gave the deoxy-compound (241) together

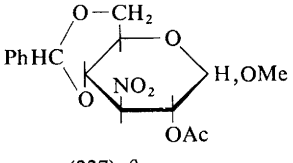
[425] R. D. Guthrie and D. Murphy, *Carbohydrate Res.*, 1967, **4**, 465.
[426] H. H. Baer, T. Neilson, and W. Rank, *Canad. J. Chem.*, 1967, **45**, 991.
[427] H. H. Baer and F. Kienzle, *J. Org. Chem.*, 1967, **32**, 3169.
[428] H. H. Baer and F. Kienzle, *Canad. J. Chem.*, 1967, **45**, 983.

with the oxime (242). The D-*talo*-isomer of (236) was similarly obtained,[428] acetylation causing dehydration directly.

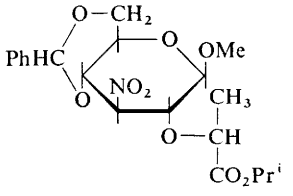
Methyl 6-deoxy-6-nitro-α- and β-D-glucopyranosides have been synthesised by treatment of the appropriate 6-deoxy-6-iodo-2,3,4-tri-*O*-tetrahydropyranyl compounds with sodium nitrite in dimethyl sulphoxide



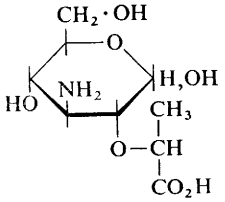
(235) β-anomer
(236) α-anomer



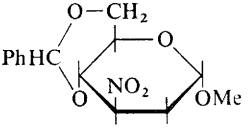
(237) β-anomer
(238) α-anomer



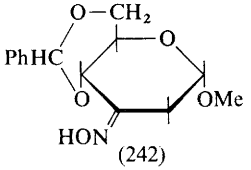
(239)



(240)



(241)



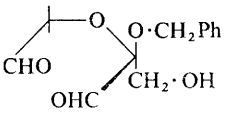
(242)

(in the presence of phloroglucinol to decompose any nitrite esters formed), followed by removal of the blocking groups.[429] The corresponding peracetates were prepared by displacement reactions on the acetylated deoxy-iodo-glycosides, but the ester groups could not be removed because of the alkaline lability of the nitro-derivatives. A similar nucleophilic displacement[429] on 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was not successful, in keeping with the known difficulty of this type of reaction.

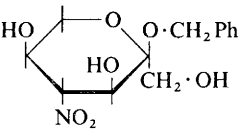
As a continuation of their studies on the nitromethane cyclisation, Lichtenthaler and his group have investigated the products from the reaction of nitromethane with the dialdehyde (243) (from benzyl β-D-fructopyranoside).[372] Two products were isolated (60 and 4% yield) and by n.m.r. studies were shown to have the α-L-*sorbo*- (244) and the β-D-*tagato*- (245) configurations. The products were reduced and converted into derivatives of the corresponding 4-amino-4-deoxyketoses.

[429] B. Lindberg and S. Svensson, *Acta Chem. Scand.*, 1967. **21**, 299.

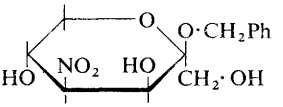
Use of the nitromethane cyclisation on the dialdehyde obtained from periodate oxidation of 3-(β-D-glucopyranosyl)thymine gave a mixture of 3-(3-deoxy-3-nitro-β-D-glycopyranosyl)thymines from which the *gluco*-isomer was isolated (25%); reduction gave the 3-amino-3-deoxy-glucosyl-nucleoside from which the free sugar was obtained on hydrolysis.[430] Similar use of the dialdehyde from 7-(β-D-glucopyranosyl)-theophylline or the



(243)



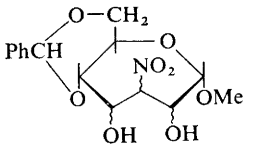
(244)



(245)

D-ribofuranosyl analogue gave a mixture of 7-(3-deoxy-3-nitro-β-D-glycopyranosyl)-theophyllines from which the *gluco*-, *manno*-, and *galacto*-isomers were isolated in yields of 24, 10, and 13% respectively.[431] Reduction gave the corresponding 3-amino-3-deoxy-hexopyranosyl-nucleosides. In a similar way 1-(3-deoxy-3-nitro-β-D-gluco- and mannopyranosyl)-cytosines have been prepared, and were reduced to the corresponding amino-derivatives.[432] Condensation of *xylo*-pentodialdose with nitroethane gave a *C*-methyl-branched inosamine derivative (see p. 160).

The nitromethane cyclisation reaction has also been applied to the dialdehyde from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside.[433] Four isomeric methyl 5,7-*O*-benzylidene-3-deoxy-3-nitro-α-D-heptoseptanosides (246) were obtained, three of which gave crystalline amino-compounds on reduction, and one of these products gave a crystalline 3-amino-3-deoxy-heptose hydrochloride. Treatment of one isomer of (246) with methanol caused the introduction of methoxy-groups at C-2 and C-4, presumably *via* unsaturated nitro-compounds.



(246)

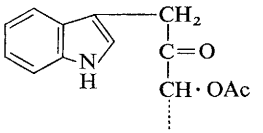
[430] F. W. Lichtenthaler and H. P. Albrecht, *Chem. Ber.*, 1967, **100**, 1845.
[431] F. W. Lichtenthaler and T. Nakagawa, *Chem. Ber.*, 1967, **100**, 1833.
[432] H. A. Friedman, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1967, **32**, 3775.
[433] M. L. Wolfrom, U. G. Nayak, and T. Radford, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **58**, 1848.

The nitromethane cyclisation reaction has been extended to the synthesis of triamino-sugars.[398b]

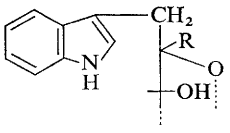
Other Nitrogen-containing Compounds

Methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-diphenylazo-α-D-mannopyranoside has been shown to be formed as a minor by-product in the reaction of periodate-oxidised methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with aqueous phenylhydrazine.[397] The diphenylazo-compound was the major product, however, if DMF was the solvent. Reaction of the same periodate-oxidised glucoside with aqueous methylhydrazine gave methyl 4,6-*O*-benzylidene-3-deoxy-3-methylazo-α-D-glucoside.[434]

Condensation of 3,4,5,6-tetra-*O*-acetyl-1-deoxy-1-diazo-derivatives of D-psicose and of D-fructose with methyl propiolate or with dimethylacetylene dicarboxylate gave pyrazole derivatives carrying a polyhydroxy-side-chain.[435] These reactions represent possible first steps in a synthesis of the *C*-nucleosides formycin and formycin B. Copper-catalysed decomposition of acetylated diazoketoses gave an oxocarbene which was trapped by a large excess of indole to give 1-deoxy-1-(indol-3-yl)-ketose acetates (247). Deacetylation with methoxide gave the parent ketose (248) but ammonia gave the glycosylamine (249).[436]



(247)



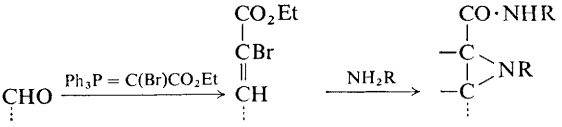
(248) R = OH
(249) R = NH_2

The product from the action of lithium aluminium hydride on methyl 3-benzamido-3,6-dideoxy-2,4-*O*-dimethanesulphonyl-α-L-glucopyranoside has been shown to be methyl 3,4,6-trideoxy-3,4-epimino-α-L-galactopyranoside by n.m.r. spectroscopy and chemical methods.[437] The 2,5-anhydro-3,4-epimino-ribose derivative (123) has been synthesised from a *trans*-azido-sulphonate precursor.[311] Acyclic epimines such as (250) have been synthesised from Wittig adducts as shown in Scheme 23.[438]

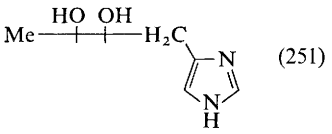
4-(*S*)-(L-*erythro*-2,3-Dihydroxybutyl)imidazole (251) has been isolated from the reaction of L-rhamnose with ammonia, suggesting that 3-deoxy-L-rhamnosone is produced as an intermediate.[439] The study of the action of

[434] E. O. Bishop, R. D. Guthrie, and J. E. Lewis, *Carbohydrate Res.*, 1967, **5**, 477.
[435] M. Sprinzl and J. Farkas, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3787.
[436] Yu. A. Zhadnov, V. I. Kornilov, and G. V. Bogdanova, *Carbohydrate Res.*, 1967, **4**, 492.
[437] A. D. Barford and A. C. Richardson, *Carbohydrate Res.*, 1967, **4**, 408.
[438] B. A. Dmitriev, N. E. Bairomova, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2691.
[439] H. Tsuchida and M. Komoto, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 185.

ammonia on acylated monosaccharides has been extended to the heptose series.[440] Derivatives of D-*glycero*-D-*gulo*-heptose, D-*glycero*-L-*manno*-heptose, and D-*glycero*-D-*galacto*-heptose were used. The hexa-acetates all gave 1,1-bis(acetamido)-1-deoxy-D-heptitols, characterised as their hexa-acetates. The yields were generally higher than in the case of hexose



Scheme 23 (250)



(251)

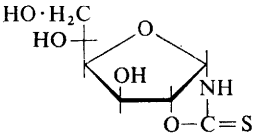
derivatives. Hexabenzoyl-D-*glycero*-L-*manno*-heptose gave the 1,1-bis (benzamido)-1-deoxy-heptitol with methanolic ammonia together with a small amount of *N*-benzoyl-D-*glycero*-L-*manno*-heptosylamine; this result is analogous to those with D-mannose and L-rhamnose (see also p. 251).

The reaction of sucrose and urea at 140° and at 220° has been studied; the products that were isolated and identified were 2-hydroxymethyl-5-methylpyrazine, 2,5-bis(hydroxymethyl)-1,4(?)-dihydroxypyrazine, 2-methyl-5-(D-*arabino*-tetrahydroxybutyl)-2-imidazolene, D-glucopyranosylurea, and *NN'*-bis(D-glucopyranosyl)urea.[441]

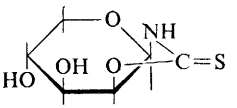
The reaction of thiocyanic acid with several free sugars has been studied.[442] D-Glucose, D-xylose, D-galactose, L-arabinose, and D-ribose all gave furanoid products, for example, (251A), whereas the D-lyxose product had the pyranose structure (252) presumably because the furanose alternative would suffer from severe steric crowding. Similarly, D-mannose did not form a furanose product, but gave, after acetylation, compound (253). D-Fructose gave two products, one cyclic (254) and one acyclic (255).

1-Arylazo-*trans*-1-hexen-D-*lyxo*-3,4,5,6-tetrol tetraacetates and their D-*arabino*-analogues have been prepared[443] with a *para* methyl, chloro-, bromo-, or iodo-group in the benzene ring, by the method previously used for the phenyl analogues. Attempts to prepare arylazo-ene derivatives from free unacetylated sugar phenylhydrazones by treatment with methanolic sodium methoxide at 120° gave glyoxal bisphenylhydrazone.

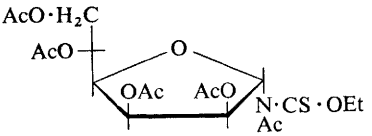
[440] J. O. Deferrari and R. M. de Lederkremer, *Carbohydrate Res.*, 1967, **4**, 365.
[441] I. Jezo and I. Luzak, *Chem. Zvesti*, 1967, **21**, 35.
[442] J. C. Jochims, A. Seeliger, and G. Taigel, *Chem. Ber.*, 1967, **100**, 845.
[443] H. El Khadem, M. L. Wolfrom, Z. M. El Shafei, and S. H. El Ashry, *Carbohydrate Res.*, 1967, **4**, 225.



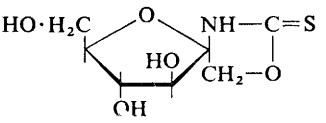
(251A)



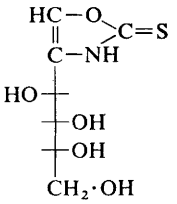
(252)



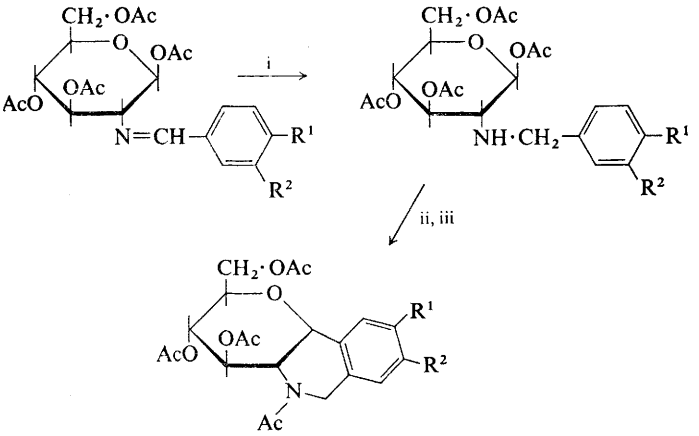
(253)



(254)



(255)

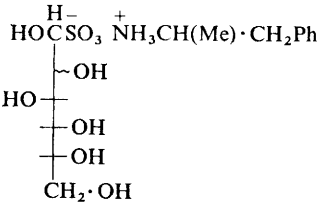


Reagents: i, H_2–Pd; ii, HCl; iii, Ac_2O–py.

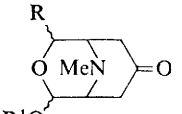
Scheme 24

A large number of substituted isoquinolines have been obtained by means of the route shown in Scheme 24.[444]

The o.r.d. spectra of a number of nitrogen heterocycles bearing polyhydroxy-side-chains have been investigated (see p. 189). Reaction of amphetamine bisulphite with D-glucose or D-mannose gave a product, believed to be (256); reaction with *N*-substituted-glycosylamines gave either the same type of product or ones in which the C-1 nitrogen function was retained.[445]



(256)



(257)

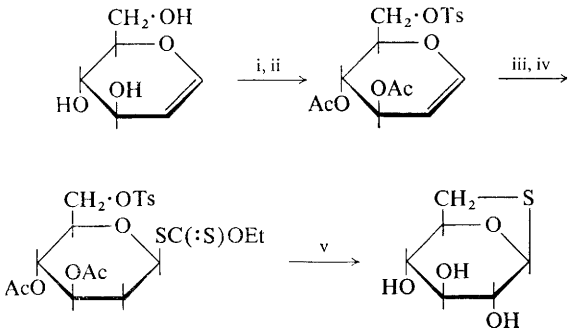
Acetylated nitriles of partially methylated D-xyloses, prepared from dehydration of the oximes, were shown to be excellent for g.l.c. characterisation purposes.[161]

Periodate-oxidised benzyl and methyl β-L-arabinopyranosides and methyl α-L-rhamnopyranoside have been used as the dialdehyde component in the Robinson–Schöpf synthesis to give analogues (257) of 9-methyl-3-oxagranat-7-one.[446]

[444] O. Wacker and H. Fritz, *Helv. Chim. Acta*, 1967, **50**, 2481.
[445] J. C. Griffin and G. S. Banker, *J. Pharm. Sci.*, 1967, **56**, 1098.
[446] R. D. Guthrie and J. F. McCarthy, *J. Chem. Soc.* (*C*), 1967, 62.

11
Thio-sugars*

Use of the neighbouring-group properties of sulphur-containing groups in sulphonyl displacements has led to the introduction of a thio-group at C-3 in D-ribose and thence to 3′-thio-adenosine,[315] and also to the synthesis of 3′-amino-3′-deoxy-2′-thio-uridine.[314] Papers continue to appear regularly from Tejima's school, who this year have described the synthesis of 2-deoxy-1,6-anhydro-1-thio-β-D-glucopyranose (2-deoxy-thiolevglucosan),[447] which was synthesised from D-glucal as shown in Scheme 25. Glycosyl *NN*-



Reagents: i, TsCl–py; ii, Ac_2O–py; iii, HBr–C_6H_6; iv, KSC(:S)OEt; v, MeONa–MeOH.

Scheme 25

dialkyldithiocarbamate peracetates have been synthesised from glycosyl bromide peracetates in the D-*gluco*-, D-*galacto*-, D-*xylo*-, and *lacto*-series by reaction with sodium *NN*-dialkyldithiocarbamates.[448] The β-configuration was assumed because of the neighbouring-group effects of the C-2-acetate group. The products could be de-acetylated with cold methanolic ammonia or sodium methoxide to give compounds that were stable to chilled alkali; they were very easily decomposed by mercuric salts in the presence of methanol to give methyl α-D-glycopyranosides, and on fusion with *p*-nitrophenol, in the presence of mercuric cyanide, they gave the *p*-nitrophenyl β-glycopyranosides. Peracetylated D-glucopyranosyl derivatives

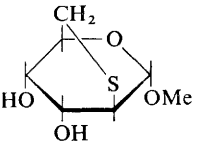
[447] T. Maki and S. Tejima, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 1367.
[448] S. Tejima and S. Ishiguro, *Chem. and Pharm. Bull.* (*Japan*), 1967, **15**, 255.

* See also Sections 6 (pp. 72–73 and 78–80) and 12: see Section 3 for *S*-glycosides.

with the *NN*-diethyldithiocarbamate group at C-6 were also prepared in which C-1 bore an acetoxy-, β-methoxy-, or *NN*-diethyldithiocarbamate group. The methanesulphonyloxy-group of 3,4,6-tri-*O*-acetyl-2-*O*-methanesulphonyl-β-D-glucopyranosyl *NN*-dialkyldithiocarbamate has been displaced by the acetate ion and by the thioacetate ion to give different types of products.[316]

The reactions of methyl 2-*S*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-2-thio-α-D-altropyranoside and of its 4,6-*O*-ethylidene-2-*S*-methyl analogue with sodium have been shown to give a variety of sulphur-containing unsaturated products (see p. 129).

In order to substantiate (cf. A. B. Foster *et al.*, *Chem. Comm.*, 1967, 759) the deshielding effect of an axial sulphoxide on the protons in *syn*-axial positions, the two sulphoxides derived from the rigid methyl 2,6-anhydro-2-thio-α-D-altropyranoside (257A) have been prepared, and the n.m.r.



(257A)

spectra of their diacetates have been studied.[449] The expected effects were observed, showing that the *syn*-axial effect is of value in assigning sulphoxide configurations.

The substance described as '6-deoxy-4-thio-L-talopyranose' has been shown [327] to be 6-deoxy-5-thio-L-talofuranose.

Pyridinium thiobenzoate has been used to open epoxides to give thiobenzoates.[186] Thirty-five sulphur-containing carbohydrate derivatives have been tested for antitumour activity. β-D-Xylopyranosyl- and β-D-mannopyranosyl-ethyl xanthate were found to have pronounced effects.[450]

A detailed n.m.r. study (including the use of 220 Mc./sec. spectra) of fully acetylated 1-thio-aldopyranoses has been made (see p. 181).

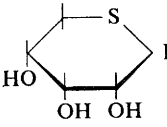
[449] A. B. Foster, J. M. Duxbury, T. D. Inch, and J. M. Webber, *Chem. Comm.*, 1967, 881.
[450] M. Akagi, S. Tejima, M. Haga, Y. Hirokawa, M. Yamada, M. Ishiguro, and D. Mizuno, *J. Pharm. Soc. Japan*, 1967, **87**, 287.

12

Derivatives with Sulphur, Selenium or Nitrogen in the Sugar Ring

Sulphur Derivatives

Full details have now been given of Hughes' work on 5-thio-D-ribopyranose (258),[451] which was synthesised by reaction of methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-β-D-ribofuranoside with potassium thioacetate in hot DMF, followed by removal of the blocking groups; the free sugar (258)



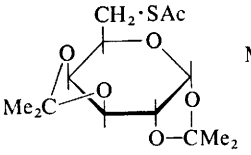
(258)

was obtained in both anomeric forms. Mutarotation of either anomer suggested that the furanose forms were absent from the equilibrium. Methanolysis gave the methyl α- and β-pyranosides as the major products: a detailed study showed that the α-anomer was formed first and that this then equilibriated with the β-anomer; the furanosides were only minor products. This behaviour was markedly different from that of D-ribose under the same conditions. As shown for other thiopyranosides, their rate of hydrolysis by aqueous acid was *ca.* ten times faster than their oxygen analogues. In contrast, it has been reported,[452] that the sulphoxide and the sulphone, derived from methyl 5-thio-β-D-xylopyranoside, were not hydrolysed by 0·5 *N*-hydrochloric acid at 75° during 100 hr.

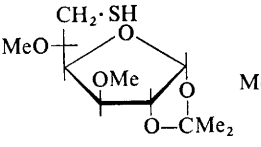
Work on the synthesis of compounds containing the thioseptanose ring has been described.[453] Compound (259) was prepared from the 6-*O*-toluene-*p*-sulphonyl or the 6-deoxy-6-iodo-derivative and was then converted by standard reactions into the D-galactosyl bromide per(*p*-nitrobenzoate), which showed *S*-ester bands in its i.r. spectrum and was not therefore a thioseptanose derivative. A second attempt involved the synthesis of (260) from 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose; when the acetal group was removed from (260) the product

[451] C. J. Clayton and N. A. Hughes, *Carbohydrate Res.*, 1967, **4**, 32.
[452] R. M. Rowell and R. L. Whistler, *Carbohydrate Res.*, 1967, **5**, 337.
[453] J. M. Cox and L. N. Owen, *J. Chem. Soc.* (*C*), 1967, 1121.

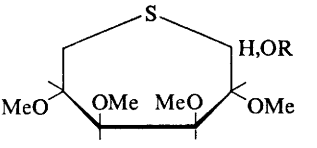
contained the —SH group and was therefore furanoid. The final attempt was to use acyclic intermediates: D-galactose dibenzyl dithioacetal was converted into 6-deoxy-6-mercapto-2,3,4,5-tetra-*O*-methyl-D-galactose ethylene acetal, which, on hydrolysis, gave the thioseptanose derivative (261); ethanolysis gave an $\alpha\beta$-mixture of ethyl thioseptanosides (262). Compound (261) reacted rapidly with iodine, that is, like a thiofuranose, but unlike thiopyranose compounds.



(259)

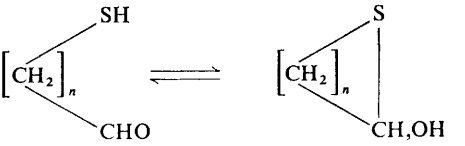


(260)



(261) R = H
(262) R = Et

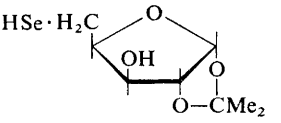
The same workers have studied model compounds for cyclic thiosugars, that is the equilibrium (263). It was shown that tautomeric change to the open-chain mercaptoaldehyde form occurred much more readily with the seven-membered ring than with the five- or six-membered rings.[454]



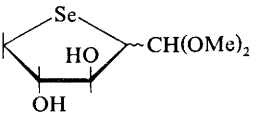
(263)

Selenium Derivatives

Reaction of 1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-xylofuranose with potassium selenocyanate in ethanol for 60 hr. have a low yield of the 5-deoxy-5-selenocyanate which, with methanolic sodium methoxide, gave a mixture of the 5-deoxy-5-selenoderivative (264) and the corresponding diselenide, which was also readily formed from (264).[455] Reduction of the selenobenzyl derivative of (264) with liquid ammonia followed by methanolysis gave three major products, one of which was the diselenide.



(264)



(265)

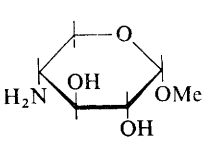
[454] J. M. Cox and L. N. Owen, *J. Chem. Soc.* (*C*), 1967, 1131.
[455] T. Van Es and R. L. Whistler, *Tetrahedron*, 1967, **23**, 2849.

The other two (both $C_7H_{14}O_4Se$) were shown to be in equilibrium in acidic methanol, and not to contain the —SeH group. Chemical and physical evidence suggested that these were C-2-epimers of 2-formyl-D-*threo*-3,4-dihydroxy-2,3,4,5-tetrahydroselenophene dimethyl acetal (265). Although not a selenoglycose, this is the first carbohydrate derivative with selenium in a ring.

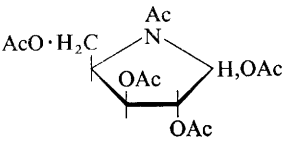
Nitrogen Derivatives

Much interest is still shown in this class of compounds, particularly by Paulsen's group at Hamburg. It has been confirmed [456] that the antibiotic nojirimycin is 5-amino-5-deoxy-D-glucose in the piperidinose form.

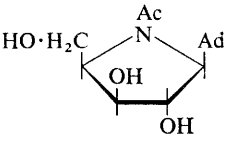
A nitrogen-containing nucleoside of this class with the D-*xylo*-configuration has been described.[248] Methyl β-L-arabinopyranoside was selectively benzoylated at C-2 and C-3, the toluene-*p*-sulphonate at C-4 was formed, and then the sulphonyloxy-group was displaced with azide ion to give, after debenzoylation and reduction, methyl 4-amino-4-deoxy-α-D-xylopyranoside (266). Acetylation of (266), followed by acetolysis, gave mainly



(266)



(267)



(268)

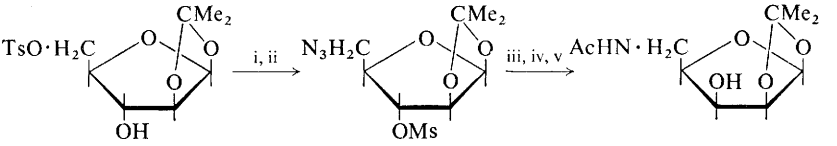
the five-membered ring compound (267) which was converted by standard reactions into the nucleoside analogue (268) in 22% yield.

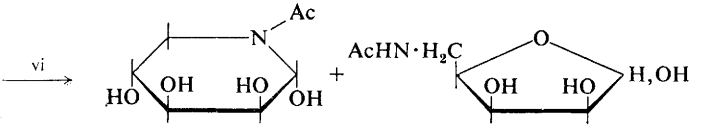
The synthesis of 5-acetamido-5-deoxy-D-lyxopyranose has been described [325] by the route shown in Scheme 26. The *N*- and *O*-heterocycles (269) and (270) were isolated in equal amounts, the former being obtained by direct crystallisation from the mixture. Compound (269) showed no mutarotation in aqueous solution and was partially isomerised to (270) upon being heated or by treatment with acids and bases. Two 1-H doublets were observed in the n.m.r. spectrum of (269), caused by the restricted rotation about the *N*-carbonyl bond.

Hydrolysis of 5-deoxy-5-hydrazino-1,2-*O*-isopropylidene-α-D-xylofuranose with sulphurous acid gave compound (271) which in alkaline solution yielded the free sugar in the piperidinose form (272),[457] which dimerised to (273). Compound (273) reacted with hydrocyanic acid to yield (274), was reduced to (275), and with hot hydrochloric acid gave the betaine (276).

[456] N. Ishida, K. Kumagai, T. Niida, T. Tsuruoka, and H. Yumoto, *J. Antibiotics* (*Japan*), *Ser. A*, 1966, **19**, 288; 1967, **20**, 66.
[457] H. Paulsen and G. Steinert, *Chem. Ber.*, 1967, **100**, 2467.

Extension to the ketose series was made by the synthesis of 6-amino-6-deoxy-2,3-*O*-isopropylidene-α-L-sorbofuranose by standard sequences from 2,3-*O*-isopropylidene-1,6-di-*O*-toluene-*p*-sulphonyl-α-L-sorbofuranose or from 1-*O*-acetyl-2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose.[458] Acid

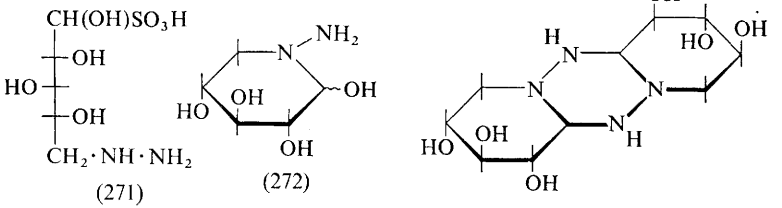




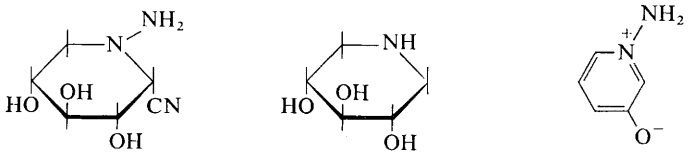
(269) (270)

Reagents: i, NaN_3–DMF; ii, MsCl–py; iii, H_2–Pd; iv, Ac_2O–py; v, NaOBz–DMF; vi, H^+.

Scheme 26



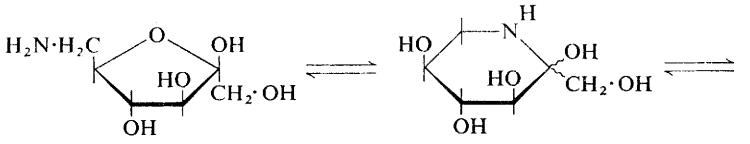
(271) (272) (273)



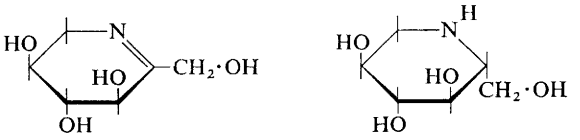
(274) (275) (276)

hydrolysis of the acetal group of the final product gave the furanose free-sugar (277), which in alkaline solution is in equilibrium with the piperidine isomer (278) and the tetrahydro-pyridine derivative (279). Hydrogenation of the last compound gave the piperidine derivative (280).

[458] H. Paulsen, I. Sangster, and K. Heyns, *Chem. Ber.*, 1967, **100**, 802.

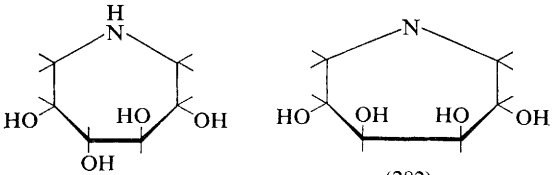


(277) (278)

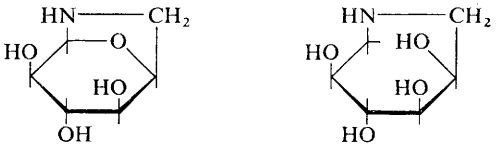


(279) (280)

Derivatives with a seven-membered nitrogen-containing ring have been prepared by an intriguing route.[459] Catalytic hydrogenation of 6-amino-6-deoxy-D-glucose and -galactose gave the seven-membered ring compounds (281) and (282). The formation of these products was interpreted as showing that the septanose form exists to at least a small degree in solution, and that this form was preferentially hydrogenated. Similarly 6-deoxy-1,6-imino-L-idopyranose (283) gave the seven-membered ring compound (284).



(281) (282)



(283) (284)

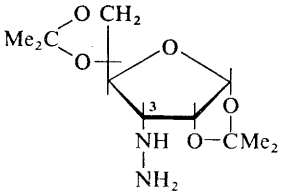
Treatment of 3-*O*-methyl-2,4-*O*-methylene-1,5-di-*O*-toluene-*p*-sulphonyl-xylitol with methanolic ammonia gave the ring-compound (165),[326] which can be considered as a derivative of a nitrogen-ring sugar.

Work has continued on the now well-established phenomenon of restricted rotation about the nitrogen–carbonyl bond of the *N*-acetyl group on the ring-nitrogen atom (see p. 183).

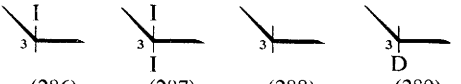
[459] H. Paulsen and K. Todt, *Chem. Ber.*, 1967, **100**, 512.

13
Deoxy-sugars

Several methods for converting hydroxy-groups to halogeno-groups have been reported that appear to have appreciable potential value in deoxy-sugar synthesis. Displacement of a sulphonyloxy-group by hydrazine gave deoxy-hydrazino-compounds which were then converted into the corresponding deoxy-halogeno-derivatives or, more usefully, directly into deoxy-sugars. 3-Deoxy-3-hydrazino-1,2:5,6-di-*O*-isopropylidene-D-allose, (285) for example, yielded the 3-deoxy-3-iodo-D-glucose derivative (286) on treatment with iodine in chloroform, iodine in aqueous potassium iodide, or *N*-iodosuccinimide; with the last two reagents, some of the 3-deoxy-3-di-iodo-derivative (287) was formed in addition. However, treatment of the

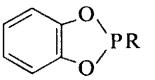


(285)



(286) (287) (288) (289)

hydrazino-compound with periodate or ferricyanide gave the 3-deoxy-compound (288) direct. The displacement occurred with retention of configuration because when the reactions were carried out in deuterium oxide the *allo*-product (289) was obtained.[460] Corey and Anderson have reported another means of converting alcohols to iodides employing *o*-phenylene phosphorochlorodite (290), a stable reagent easily prepared from catechol and phosphorus trichloride. In ether solution this reacted

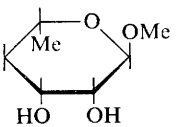


(290) R = Cl
(291) R = OAlkyl
(292) R = OI

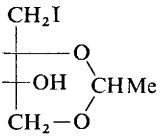
with an alcohol in the presence of pyridine to give the corresponding phosphite (291) which, in methylene dichloride, gave the alkyl iodide on treatment with iodine. Isolation of the required product is facilitated by

[460] D. M. Brown and G. H. Jones, *J. Chem. Soc.* (*C*), 1967, 252.

the rapid base-catalysed hydrolysis of the *o*-phenylene iodophosphate (292) which is also formed. Although the reaction has not yet been applied to carbohydrate derivatives, the report that cyclohexyl iodide can be prepared from cyclohexanol in 95% yield would appear to suggest that this might represent a simple and efficient procedure.[461] Triphenylphosphite methiodide, however, is known to be applicable to such conversions and has been used in the synthesis of methyl 4,6-dideoxy-α-L-*lyxo*-hexoside (293) from methyl 2,3-*O*-isopropylidene-α-L-rhamnoside.[462]



(293)



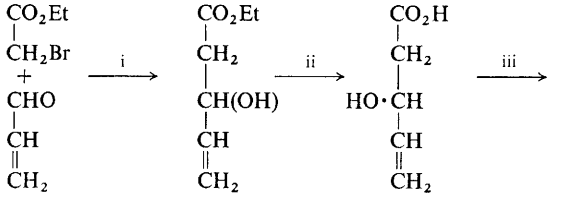
(294)

Several other syntheses of deoxy-sugars and their derivatives have been reported. Ziderman and Dimant have developed a synthesis of 2-deoxy-D-*erythro*-pentose which has the advantage of being applicable to the preparation of the C-1 specifically labelled sugar. Treatment of 1-deoxy-2,4-*O*-ethylidene-1-iodo-D-erythritol (294) with sodium cyanide in DMSO at 37° gave the pentononitrile which on reduction with excess of Raney nickel in aqueous acetic acid, and hydrolysis gave the 2-deoxy-pentose in 27% yield. Use of 2,3,4-tri-*O*-benzoyl-1-bromo-(or chloro- or iodo-)1-deoxy-D-erythritol in this type of reaction did not meet with success.[463] Total syntheses of 2-deoxy-DL- and -L-*erythro*-pentose have been reported, using the route shown in Scheme 27. Resolution of the acid (295) gave the (−) form which was used in the synthesis of the L-derivative.[464] Another 2-deoxy-aldose, the *manno*-heptose sugar (296), has been synthesised to aid in the identification of a compound obtained from 3-deoxy-D-*manno*-octulosonic acid which is a component of the cell-wall lipoglycan of *Escherichia coli*. 1-Deoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (297) was acetylated and converted to the nitro-olefin which on hydrogenation and subjection to the Nef reaction afforded the required heptose.[465]

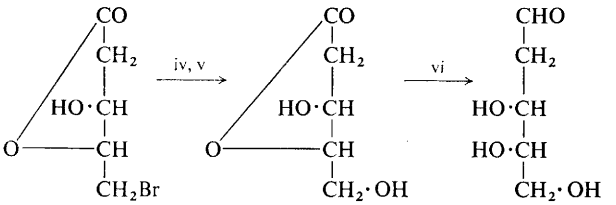
Selective hydrolysis of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexose followed by periodate oxidation and borohydride reduction gave 3-deoxy-1,2-*O*-isopropylidene-D-*erythro*-pentose (298), which, together with the D-*threo*-isomer, was also synthesised by the following route.[466]

[461] E. J. Corey and J. E. Anderson, *J. Org. Chem.*, 1967, **32**, 4160.
[462] K. S. Adamyants, N. K. Kochetkov, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 1311.
[463] I. Ziderman and E. Dimant, *J. Org. Chem.*, 1967, **32**, 1267.
[464] G. Nakaminami, M. Nakagan, S. Shioi, Y. Sugiyama, S. Isemure, and M. Shibuya, *Tetrahedron Letters*, 1967, 3983.
[465] M. B. Perry, *Canad. J. Chem.*, 1967, **45**, 1295.
[466] D. M. Brown and G. H. Jones, *J. Chem. Soc.* (*C*), 1967, 249.

Partial hydrolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucose and periodate oxidation of the product gave an aldehyde which eliminated toluene-*p*-sulphonic acid on treatment with sodium methoxide

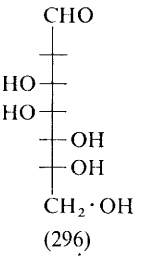


(295)

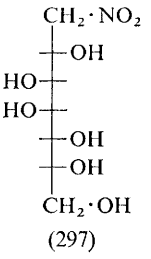


Reagents: i, Zn; ii, aq. KOH; iii, NBS–H_2O; iv, aq. KOH; v, H^+; vi, HB(Pent^i).

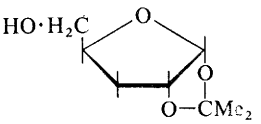
Scheme 27



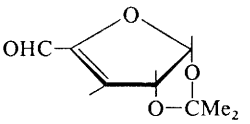
(296)



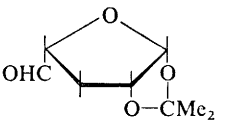
(297)



(298)



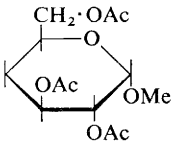
(299)



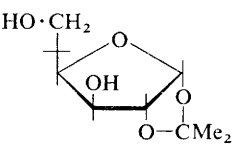
(300)

to give the unsaturated aldehyde (299) (together with some methanol adduct). Selective reduction of this gave the saturated aldehyde (300) which could be equilibrated with the D-*erythro*-isomer. Reduction of this mixture with borohydride gave compound (298) and its 4-epimer in the ratio 1 : 4.

Methyl 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranoside (301) (and consequently '4-deoxy-glucose') has been shown to be readily available by displacement of the sulphonyloxy-group from methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methanesulphonyl-α-D-galactopyranoside by thiocyanate ion in DMF and subsequent standard conversions.[329]



(301)



(302)

A report on the preparation of '5-deoxy-glucose' has also appeared which clears up some confusion surrounding this compound.[467] The Raney nickel-catalysed hydrogenation of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose was studied in detail, but the best route into the 5-deoxy-series involved lithium aluminium hydride treatment of 6-*O*-benzoyl-1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose to give compound (302), together with a little of the 6-deoxy-L-idofuranose isomer.

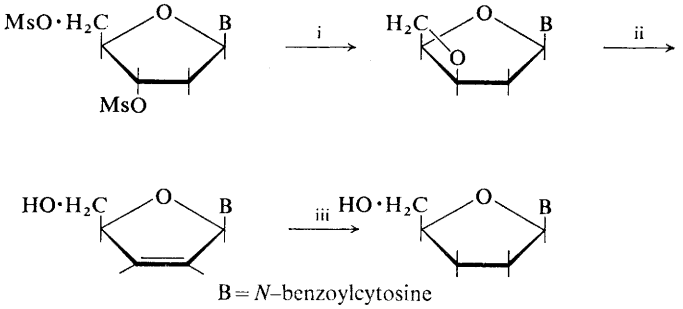
In the course of their extensive examination of the deoxy-sugars of plant glycosides, Reichstein and his colleagues have prepared 6-deoxy-L-idose and the related ketose 6-deoxy-L-sorbose, and have found them to be distinguishable by chromatography and electrophoresis, although their melting points and optical rotations were similar. 6-Deoxy-L-psicose was also described.[468] In another paper the same authors recorded the paper chromatographic, t.l.c., and electrophoretic mobilities of all the 6-deoxy-hexoses and their 3-*O*-methyl ethers and all the 6-deoxy-hexuloses. All are distinguishable from their isomers.[469] 6-Deoxy-2-*O*-methyl-D-allose (javose) has been synthesised.[155]

Nucleosides containing unsaturation in the sugar moiety and dideoxy-nucleosides obtainable from them have attracted attention because of their potential significance as chain terminators of DNA biosynthesis. 2′,3′-Dideoxycytidine has now been synthesised as shown in Scheme 28.[470] The chemical shifts for the carbohydrate protons of several 3′,5′-anhydro-2′-deoxy-nucleosides have been reported.[470] Other deoxy-nucleosides are described in Section 21, and deoxy-compounds derived from unsaturated sugars are referred to in Section 14. The periodate oxidation of deoxy-sugars is described on p. 172.

N.m.r. studies have shown that the α- and β-anomers of 1,3,4-tri-*O*-benzoyl-2-deoxy-D-*erythro*-pentopyranose exist in the chair conformations

[467] E. J. Hedgley, O. Mérész, and W. G. Overend, *J. Chem. Soc.* (*C*), 1967, 888.
[468] H. Kaufmann and T. Reichstein, *Helv. Chim. Acta*, 1967, **50**, 2280.
[469] H. Kaufmann, P. Muhlradt, and T. Reichstein, *Helv. Chim. Acta*, 1967, **50**, 2287.
[470] J. P. Horwitz, J. Chua, M. Noel, and J. T. Donatti, *J. Org. Chem.*, 1967, **32**, 817.

in which the C-1 groupings are axial (*C*1 and 1*C*, respectively),[349] and o.r.d. measurements on a number of 2-deoxy-glycosides have been recorded.[150]

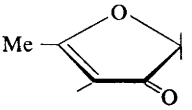


B = *N*–benzoylcytosine

Reagents: i, aq. NaOH; ii, KOBu^t–DMSO; iii, H_2–Pd.

Scheme 28

The chromophore produced by acid degradation of 2-deoxy-D-*erythro*-pentose which has provided means for the analysis of this sugar in DNA has been isolated and characterised by spectroscopic and chemical means as 5-methyl-3(2*H*)-furanone (303).[42a, b] A mechanism for the degradation



(303)

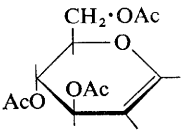
was suggested involving eliminations from the furanose form of the pentose, but is seems more probable to the Reviewers that eliminations would precede cyclisation.

14
Unsaturated Derivatives*

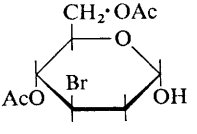
Carbohydrates containing carbon–carbon double bonds represent one of the active growth points in the field, and in 1967 appreciable developments were reported.

Glycals

Several new features of addition reactions to glycals have been observed, for example the product resulting from the addition (first described by Fischer) of hydrogen bromide in acetic acid to 3,4,6-tri-*O*-acetyl-D-glucal (304) has been shown[471] to be 4,6-di-*O*-acetyl-3-bromo-2,3-dideoxy-α-D-*arabino*-hexopyranose (305) which presumably was formed by addition,



(304)



(305)

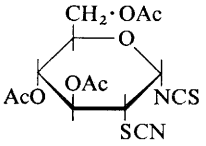
subsequent to an allylic displacement reaction of a known type. This accounts for Fischer's observation of a non-ionisable C—Br bond, which he explained by allocation of the (unlikely) 2-bromo-2-deoxy-structure to the product.

The same glycal ester has been treated, for the first time, with thiocyanogen in a mixture of acetic acid, acetic anhydride, and carbon tetrachloride and gave mainly 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-thiocyanato-D-glucose and -mannose, that is, the reaction effectively caused the addition of the acetoxy- and thiocyanato-groups at C-1 and C-2, respectively. In addition, however, small amounts of 3,4,6-tri-*O*-acetyl-2-deoxy-2-thiocyanato-α-D-glucopyranosyl isothiocyanate (306) and 3,4,6-tri-*O*-acetyl-2-thiocyanato-D-glucal (307) were isolated.[472] A series of reactions was carried out on the major products which resulted in the preparation of,

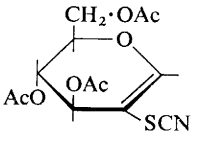
[471] T. Maki and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 1069
[472] K. Igarashi and T. Honma, *J. Org. Chem.*, 1967, **32**, 2521.

* Ascorbic acid is discussed in Section 17.

amongst several compounds, methyl 2-deoxy-α- and β-D-*arabino*-hexopyranosides and methyl 2-thio-D-gluco- and mannopyranoside derivatives, respectively. Another 2-*S*-substituted glycal has been described,[316] and also substituted 2-amino-glycals.[378–380]

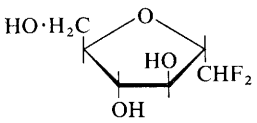


(306)

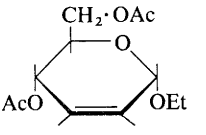


(307)

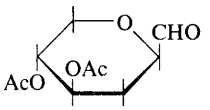
A full report of the reaction of lead tetra-acetate in anhydrous hydrogen fluoride with tri-*O*-acetyl-D-glucal (304) has been published; the main product (308), isolated after deacetylation, was also obtained on similar treatment of the 2,3-unsaturated glycoside (309).[473] Another previously



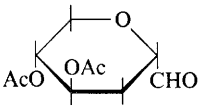
(308)



(309)



(310)



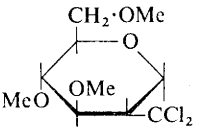
(311)

described reaction which has been extended is the 'oxo' reaction as applied to glucal derivatives. Whereas earlier experiments resulted in 2,6-anhydro-3-deoxy-alditol derivatives (addition of H, CH_2OH), details have now been given for the synthesis of the direct products of hydroformylation.[474] The C-2 epimers, 4,5-di-*O*-acetyl-2,6-anhydro-3-deoxy-*aldehydo*-D-*xylo*- and D-*lyxo*-hexoses (310) and (311), were isolated in 25% combined yield, from 3,4-di-*O*-acetyl-D-xylal and analogous products were obtained in 70% yield from tri-*O*-acetyl-D-glucal (304). A more satisfactory means for obtaining the formyl compounds, however, was to subject the fully reduced hydroxymethyl derivatives to oxidations in DMSO.

[473] K. R. Wood and P. W. Kent, *J. Chem. Soc.* (*C*), 1967, 2422.
[474] A. Rosenthal, D. Abson, T. D. Field, H. J. Koch, and R. E. J. Mitchell, *Canad. J. Chem.*, 1967, **45**, 1525.

In developments of older work, Japanese authors have treated 3,4-di-*O*-acetyl-D-arabinal with adenine in DMSO in the presence of hydrochloric acid, and have produced 9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-*erythro*-pentosyl)-adenine in 7% yield after separation on alumina. The position of substitution was established by the usual u.v. spectroscopic method, and deacetylation afforded the known 2′-deoxypyranosyl-nucleoside.[475]

The first full report of the addition of carbenes to unsaturated sugars has appeared; addition of dichlorocarbene to 3,4,6-tri-*O*-methyl-D-glucal gave a product tentatively described as 1,5-anhydro-2-deoxy-1,2-*C*-(dichloromethylene)-3,4,6-tri-*O*-methyl-D-*glycero*-D-*ido*-heptitol (312).[476] (Similar treatment of a 3,4-unsaturated sugar is mentioned below.)



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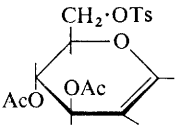
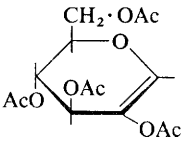


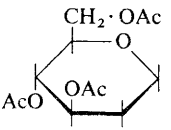
(313)

2-Deoxy-1,6-anhydro-1-thio-β-D-*arabino*-hexopyranose has been synthesised from D-glucal.[447] The addition of hydrogen bromide to the glycal ester (313) in benzene solution occurred directly[447] rather than by the indirect manner mentioned above.[471]

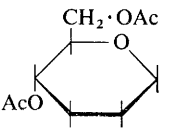
An efficient means of reductively removing halogen from acylglycosyl halides to give 1,5-anhydroalditols has been developed; platinum catalysts in dry ethyl acetate containing diethylamine were best. Elimination of hydrogen halide occurred to some extent with the resultant formation of hydroxy-glycal esters which, in turn, were reduced to give by-products. In order that these could be characterised the hydrogenation of tetra-*O*-acetyl-2-hydroxy-D-glucal (314) was examined in detail. Platinum catalysts caused complete hydrogenolysis of the vinyl acetate group together with



(314)



(315)



(316)

some cleavage of the allylic ester group prior to saturation of the double bond, so that the anhydro-compounds (315) and (316) were the sole products of reduction. When diethylamine was present allylic cleavage was promoted and compound (316) was formed almost exclusively.[198] Palladium

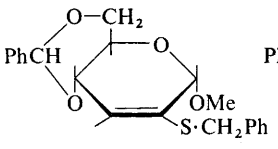
[475] N. Nagasawa, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, 1967, **32**, 251.
[476] J. S. Brimacombe, M. E. Evans, E. J. Forbes, A. B. Foster, and J. M. Webber, *Carbohydrate Res.*, 1967, **4**, 239.

catalysts, on the other hand, catalysed hydrogenation more effectively than hydrogenolysis, and 1,5-anhydro-mannitol and -glucitol acetates were formed in high yield with the former predominating. Similar studies were carried out on the hydrogenation of tri-*O*-acetyl-D-glucal (304) and again it was found that diethylamine promoted allylic hydrogenolysis, so that in its presence (316) again predominated amongst the products. N.m.r. spectra of derivatives of 1,5-anhydro-D-glucitol were discussed.[198]

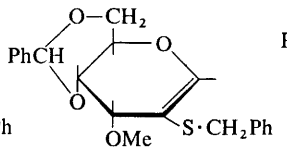
Other Unsaturated Compounds

Treatment of epoxide-containing compounds with potassium selenocyanate in methanol has provided yet another means of introducing double bonds into carbohydrates; under some circumstances α-hydroxy-selenocyananates were produced but these could also be converted into olefins *via* sulphonyl derivatives. Acyclic vicinal di-*O*-toluene-*p*-sulphonyl esters also underwent elimination on treatment with potassium selenocyante in DMF.[477]

Elimination occurred when methyl *S*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-2-thio-α-D-altropyranoside was treated with sodium in liquid ammonia in the presence of dimethoxyethane and the unsaturated compound (317) was isolated. When, however, dimethoxyethane was employed



(317)



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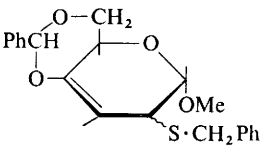


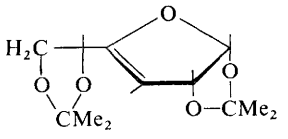
(319)

alone the three isomers (317), (318), and (319) were obtained; analogous unsaturated glycosides were produced when the benzyl group of the starting material was replaced by methyl.[478] I.r. and n.m.r. spectral characteristics were given for all the products. In a second report the same authors recorded the formation of other three analogous compounds on similar treatment of methyl 4,6-*O*-ethylidene-3-*OS*-dimethyl-2-thio-α-D-altropyranoside. Again full n.m.r. details were given.[479]

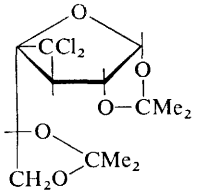
Dichlorocarbene has been added across the double bond of compound (320)[476] (see also above), and the product has been shown, by *X*-ray crystallographic methods[480] to be the *galacto*-derivative (321).

Work on the 3-acetoxy-derivative (323) of the unsaturated compound (320) has led to a practical synthesis of D-gulose.[481] Oxidation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose gave a product which crystallised

[477] T. Van Es, *Carbohydrate Res.*, 1967, **5**, 282.
[478] U. G. Nayak, M. Sharma, and R. K. Brown, *Canad. J. Chem.*, 1967, **45**, 481.
[479] U. G. Nayak, M. Sharma, and R. K. Brown, *Canad. J. Chem.*, 1967, **45**, 1767.
[480] J. S. Brimacombe, P. A. Gent, and T. A. Hamor, *Chem. Comm.*, 1967, 1305.
[481] W. Meyer zu Reckendorf, *Angew. Chem. Internat. Edn.*, 1967, **6**, 177.



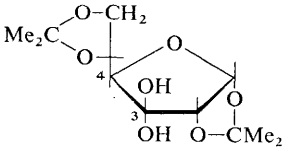
(320)



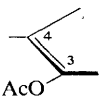
(321)

as the hydrate (322) and this, on acetylation with pyridine and acetic anhydride, suffered an elimination to give the enol acetate (323) (81%) which with sodium borohydride in methanol yielded the D-*gulo*-isomer (324) of the starting material (60%). The 5,6-acetal group of the vinyl ester was labile in alkali and was removed by the sodium borohydride in a side-reaction.

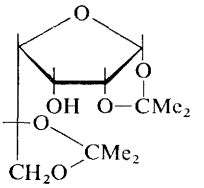
Several 2,3-unsaturated cyclic derivatives have been reported. 2,5-Anhydro-3,4-di-*O*-toluene-*p*-sulphonyl-D-xylose and several derivatives



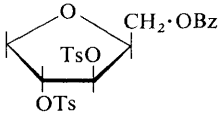
(322)



(323)



(324)



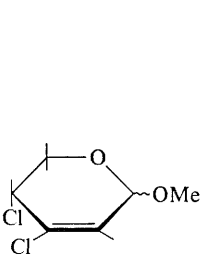
(325)

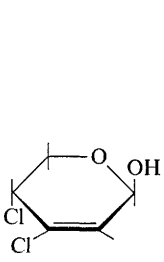
have been described, and the benzoate (325) has been converted into the olefin (117) as expected, on treatment with sodium iodide in DMF in the presence of zinc dust.[482] The same reaction has been described for the *ribo*-isomer of (325).[309a] Compounds (116)[308] and (121)[309b] have also been prepared by this method. Pyranoid compounds are better known, and the reactions of methyl 2,3-dideoxy-3-nitro-β-D-hex-2-enopyranosides have already been described in this Report.[398, 426–428]

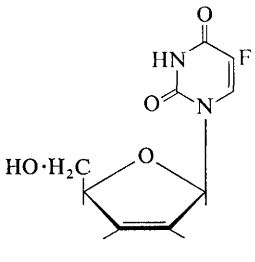
An interesting, detailed n.m.r. study has been made of methyl 3,4-dichloro-2,3,4-trideoxy-α- and β-D-*glycero*-pent-2-enopyranoside (326) and

[482] J. Cleophax, J. Hildesheim, and S. D. Gero, *Compt. rend.*, 1967, *C*, **265**, 257.

3,4-dichloro-2,3,4-trideoxy-β-D-*glycero*-pent-2-enopyranose (327) which were prepared from products obtained from the chlorosulphation of L-arabinose. The glycosides existed in opposite half-chair conformations


(326)

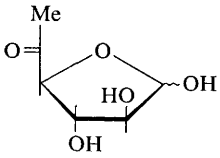

(327)

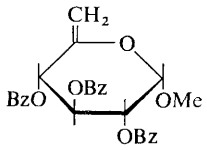

(328)

each having the methoxy-group in the *quasi*-axial orientation. The β-glycoside, which also has the groups at C-1 and C-4 *quasi*-axial, was the more stable thermodynamically; this was interpreted in terms of dipolar interactions and Van der Waals' forces.[483]

The unsaturated nucleoside (328) was prepared from the 2,3′-cyclo-2-deoxy-*ribo*-precursor, and from it (328), a 5′-phosphate was obtained which showed marked biological activity.[484]

Compounds with terminal exocyclic double bonds on hydrolysis afford deoxy-ketoses, and this reaction has been used to synthesise 6-deoxy-D-*arabino*-hexofuranose-5-ulose (329), the carbohydrate component of


(329)

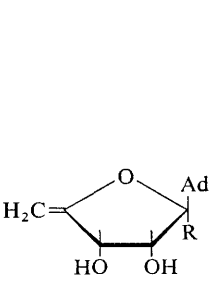

(330)

hygromycin A. Compound (330) prepared from the 6-deoxy-6-iodo-derivative by de-esterification and acid hydrolysis gave the required sugar.[485] The acetyl analogue of (330) can be labelled in the 6-position as well as in the acetyl groups by treatment with silver fluoride and pyridine in the presence of traces of tritiated water.[486]

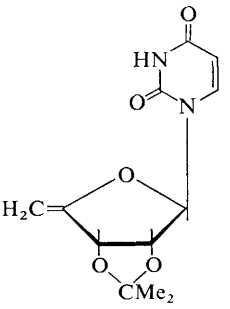
Nucleosides with this type of structure have been found in natural products and this has initiated work on their synthesis. 6-Amino-9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)purine (331) has been prepared as

[483] B. Coxon, H. J. Jennings, and K. A. McLauchlan, *Tetrahedron*, 1967, **23**, 2395.
[484] T. A. Khwaja and C. Heidelberger, *J. Medicin. Chem.*, 1967, **10**, 1066.
[485] S. Takahashi and M. Nakajima, *Tetrahedron Letters*, 1967, 2285.
[486] J. Lehmann, *Carbohydrate Res.*, 1967, **4**, 196.

an analogue of angustmycin A (332) from 2′,3′-*O*-ethoxymethylidene-5′-*O*-toluene-*p*-sulphonyladenosine by treatment with potassium *t*-butoxide in t-butyl alcohol followed by removal of the blocking groups.[487] The related nucleoside (333) was prepared by similar methods, and also by



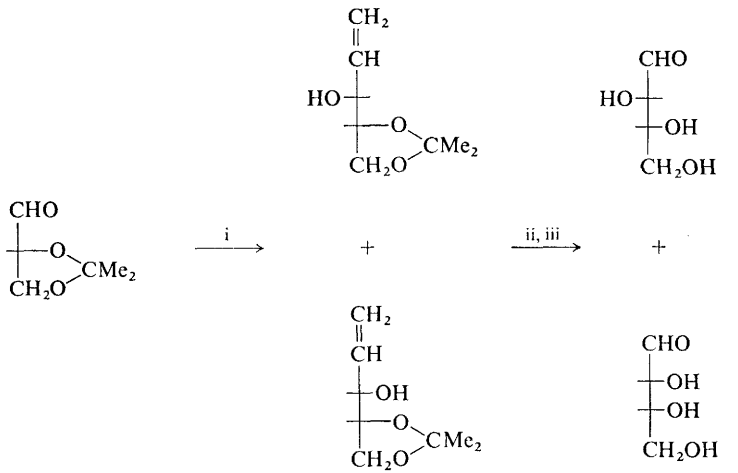
(331) R = H
(332) R = $CH_2 \cdot OH$



(333)

treatment of the appropriate 2,5′-anhydronucleoside derivative with potassium t-butoxide in DMSO.[488] Catalytic reduction and partial hydrolysis gave the 5′-deoxy-L-*lyxo*-compound with better than 90% stereoselectivity. The 5′-deoxy-*ribo*-isomer was prepared for comparative purposes.[488]

Grignard and Wittig reactions have been employed to give chain-extended carbohydrate compounds containing carbon–carbon double



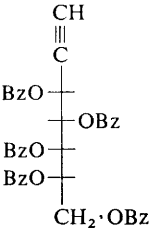
Reagents: i, CH_2=CHMgCl; ii, Ozonolysis; iii, H^+.

Scheme 29

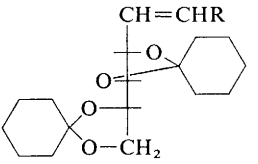
[487] J. R. McCarthy, jun., M. J. Robins, and R. K. Robins, *Chem. Comm.*, 1967, 536.
[488] M. J. Robins, J. R. McCarthy, jun., and R. K. Robins, *J. Heterocyclic Chem.*, 1967, **4**, 313.

bonds. By a simple application of the former reaction, a new synthesis of aldotetroses has been devised as shown in Scheme 29; the products of the addition were separated by preparative g.l.c.[489]

In a related experiment, Horton and his co-workers have shown that the chain-extension method which employs ethynylmagnesium bromide and *aldehydo*-derivatives could be used successfully in the presence of benzoyl protecting groups. 2,3,4,5-Tetra-*O*-benzoyl-*aldehydo*-L-arabinose gave (after benzoylation) a separable mixture of 3,4,5,6,7-penta-*O*-benzoyl-1-heptyn-L-*gluco*-3,4,5,6,7-pentol (334) and the L-*manno*-epimer. The debenzoylated products were separable as their TMS derivatives on gas chromatograms and were not epimerised by sulphuric acid. Further experiments showed that the acetylenic group was stable to basic and acid conditions, so that many of the common protecting groups could be removed in its presence.[490]

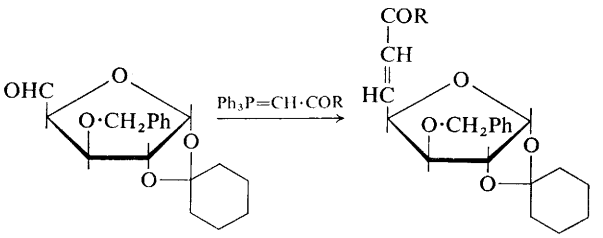


(334)



(335) R = Ph
(336) R = CH = CH_2

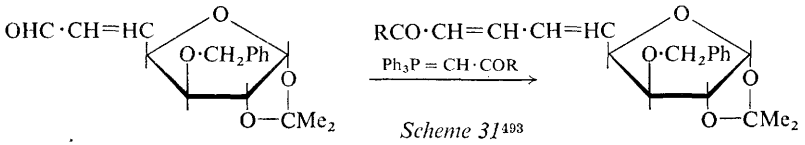
Several groups of Russian workers have published on applications of the Wittig reaction, and have shown that a wide variety of chain-extended carbohydrate derivatives were attainable by this process. Phenyl and vinyl Wittig reagents with 2,3:4,5-di-*O*-cyclohexylidene-L-arabinose gave the products (335) and (336).[491] More complex applications are illustrated in Schemes 30–32. This last reaction provides a route to 3-deoxy-aldulosonic



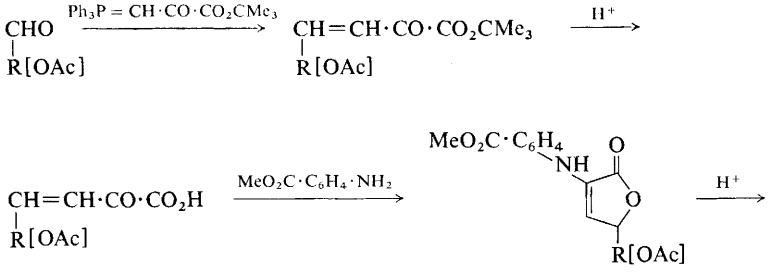
Scheme 30[492]

[489] D. J. Walton, *Canad. J. Chem.*, 1967, **45**, 2921.
[490] J. L. Godman, D. Horton, and J. M. J. Tronchet, *Carbohydrate Res.*, 1967, **4**, 392.
[491] Yu. A. Zhadnov and V. G. Alekseeva, *Zhur. obshchei Khim.*, 1967, **37**, 1408.
[492] Yu. A. Zhadnov, Yu. E. Alekseev, and G. N. Dorofeenko, *Zhur. obshchei Khim.*, 1967, **37**, 98.

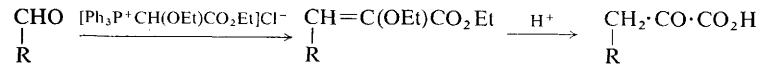
acids and also a method of ascending the aldose series.[494, 495] Similarly, 3-deoxy-aldulosonic acids may be prepared using phosphonium salts as shown in Scheme 33.[496] The addition of Wittig reagents to 2-amino-2-deoxy-D-glucose has already been described.[368]



Scheme 31[493]



Scheme 32[494]



Scheme 33[496]

[493] Yu. A. Zhadnov, Yu. E. Alekseev, and G. N. Dorofeenko, *Zhur. obshchei Khim.*, 1967, **37**, 2635.

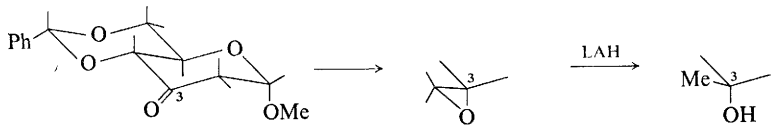
[494] N. K. Kochetkov, B. A. Dmitriev, and L. V. Backinowsky, *Carbohydrate Res.*, 1967, **5**, 399.

[495] B. A. Dmitriev, N. E. Bairamova, L. V. Bakinovskii, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1967, **173**, 350.

[496] M. N. Mirzayanova, L. P. Davydova, and G. I. Samokhvalov, *Doklady Akad. Nauk S.S.S.R.*, 1967, 173, 367.

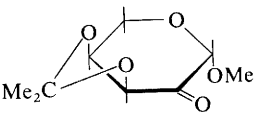
15
Branched-chain Sugars

A preliminary report has appeared on the reaction of dimethylsulphoxonium methylide with some aldulose derivatives to give *spiro*-epoxides that were then reduced with lithium aluminium hydride to give branched-chain compounds,[497] as in Scheme 34. With the same reagent, methyl 3,4-*O*-



Scheme 34

isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose (337) gave a mixture of epoxides which, when treated with alkali and then acid, yielded methyl 2-*C*-hydroxymethyl-β-L-ribopyranoside and its C-2-epimer. Reduction of this same epoxide mixture gave, after removal of the acetal group, methyl

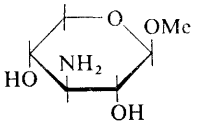


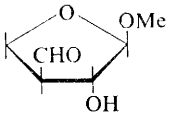
(337)

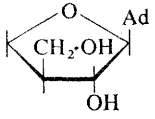
2-*C*-methyl-β-L-arabinopyranoside as the major product (75% of mixture). 5-*O*-Benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuran-3-ulose was also epoxidised with the same reagent.

A new approach to the synthesis of branched-chain nucleosides has been briefly described.[498] Nitrous acid deamination of methyl 3-amino-3-deoxy-β-D-xylopyranoside (338) occurred with ring-contraction to give the branched-chain compound (339) which was reduced, esterified, and converted into the glycosyl halide by standard sequences, which in turn was converted into the branched-chain nucleoside (340). Formation of a cyclo-nucleoside proved the β-configuration.

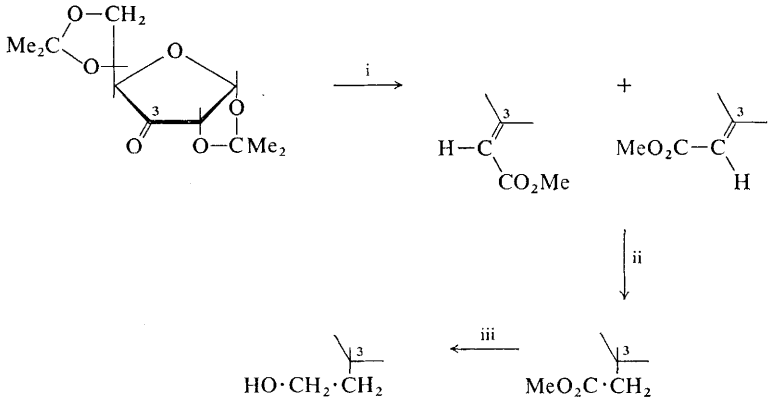
[497] R. D. King, W. G. Overend, J. Wells, and N. R. Williams, *Chem. Comm.*, 1967, 726.
[498] E. J. Reist, *Chem. and Ind.*, 1967, 1957.


(338)


(339)


(340)

The use of a modified Wittig reaction on aldulose derivatives as a route to branched-chain sugars has been described,[499] as shown in Scheme 35. The olefinic derivatives were formed in the ratio 1 : 3 but it was not shown which was which.



Reagents: $(MeO)_2P(:O)CH_2 \cdot CO_2Me$–DMF, Bu^tOK; ii, H_2–Pd; iii, LAH.

Scheme 35

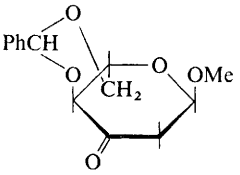
The addition of methylmagnesium bromide to blocked carbohydrates containing terminal PhCO-groups, or the addition of phenylmagnesium bromide to one with the MeCO-group was stereospecific and permitted the synthesis of benzylic centres of known absolute stereochemistry.[500] Such groupings may then be removed from their carbohydrate framework and attached to other systems.

Several syntheses of the naturally occurring branched-chain sugars, mycarose and cladinose, have been reported and a brief description of the preparation of the L-sugars has now appeared.[501] The method, based on standard sequences, starts from compound (341), the D-isomer of which has been described previously; improvements in the experimental procedure were given.

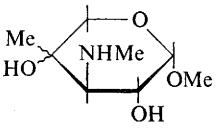
Two new naturally occurring branched-chain sugars have been found as components of antibiotics. The action of methanolic hydrogen chloride

[499] A. Rosenthal and L. Nguyen, *Tetrahedron Letters*, 1967, 2393.
[500] T. D. Inch, R. V. Ley, and P. Rich, *Chem. Comm.*, 1967, 865.
[501] G. B. Howarth and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 2253.

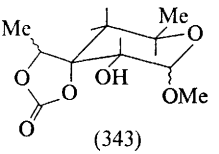
on the broad-spectrum antibiotic gentamicin C (from fermentations of *Micromonospora*) gave a methyl glycoside designated as methyl garosaminide, isolated as an α–β mixture. Evidence described in a preliminary report enabled the partial structure (342), or its enantiomer, to be assigned to the α-anomer.[352] The full account[502] of the structural studies on the products from the methanolysis of aldgamycin E (from *Streptomyces*



(341)

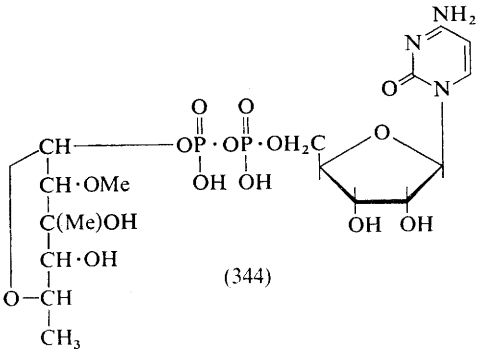


(342)



(343)

lavendulae), namely methyl aldgarosides A and B, has now appeared (the other methanolysis product, methyl mycinoside which is obtained similarly from chalcomycin and neutramycin, had been characterised by other workers). Since the publication of the preliminary account the structures have been revised and are now shown to be the anomeric branched-chain carbonates (343).



(344)

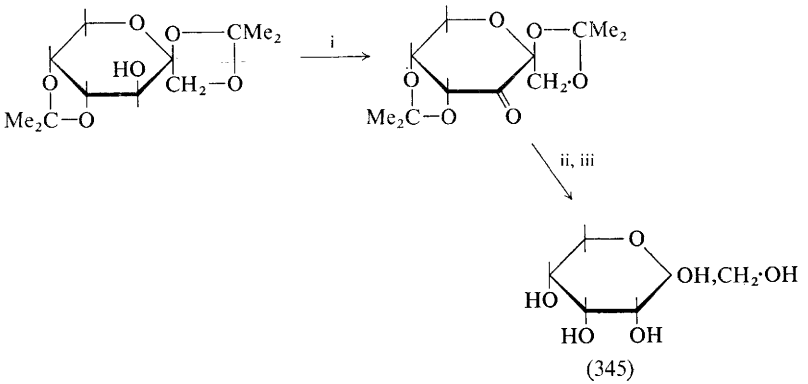
A new naturally occurring branched-chain sugar has been found attached to a cytidine nucleotide from *Azobacter vinelandii*, to which structure (344) has been assigned, and has been shown to be a 6-deoxy-3-*C*-methyl-2-*O*-methyl-L-aldohexose.[503] The limited quantities of material available did not permit the assignment of the configurations at C-1, C-2, C-3, and C-4. A new isoflavone glycoside from *Dalbergia lanceolaria*, named lanceolarin, has been shown to be the 7-apiosyl-glucoside of biochanin-A.[137]

[502] G. A. Ellestad, M. P. Kuntsmann, J. E. Lancaster, L. A. Mitscher, and G. Morton, *Tetrahedron*, 1967, **23**, 3893.
[503] S. Okuda, N. Suzuki, and S. Suzuki, *J. Biol. Chem.*, 1967, **242**, 958.

16
Alduloses and Diuloses*

The use of reagents based on DMSO for the synthesis of these compounds continues to be widely reported and a general review has appeared.[504]

Oxidations of 1,2:4,5-di-*O*-isopropylidene-D-fructopyranose[505, 506] and of the analogous cyclohexylidene diacetal[506] with DMSO and acetic anhydride have been described as an intermediate step in a new ready synthesis of D-psicose (345) (Scheme 36) which was produced by sodium boro-



(345)

Reagents: i, DMSO–Ac_2O; ii, $NaBH_4$[505, 506] or LAH[506]; iii, H^+.

Scheme 36

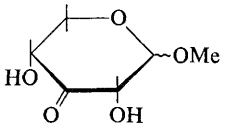
hydride[505, 506] or lithium aluminium hydride[506] reduction of the ketulose diacetal, followed by acid hydrolysis. Oxidation[506] of the above two acetals and of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose was accompanied by formation of the methylthiomethyl ether of the starting alcohol.

Methyl α- and β-D-*erythro*-pentopyranosid-3-uloses (346) have been prepared[507] in crystalline form, in good yield, by the oxidation of the anomeric methyl D-xylopyranoside 2,4-phenylboronates with DMSO and

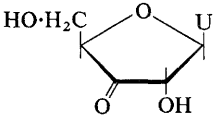
[504] W. W. Epstein and F. W. Sweat, *Chem. Rev.*, 1967, **67**, 247.
[505] E. J. McDonald, *Carbohydrate Res.*, 1967, **5**, 106.
[506] K. James, A. R. Tatchell, and P. K. Ray, *J. Chem. Soc.* (*C*), 1967, 2681.
[507] B. Lindberg and K. N. Slessor, *Acta Chem. Scand.*, 1967, **21**, 910.

* See also Section 22.

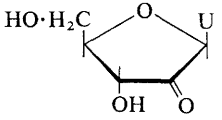
acetic anhydride, followed by removal of the blocking group and chromatography of the products on ion-exchange resins in the bisulphite form. This technique was advocated for the purification of aldulose derivatives and for the separation of aldoses and ketoses.



(346)



(347)



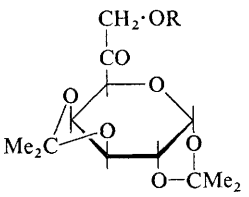
(348)

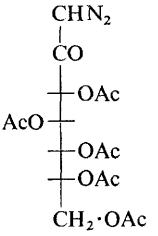
Oxidation of 2′,5′- and 3′,5′-di-*O*-trityluridine with DMSO in the presence of phosphorous pentoxide or acetic anhydride, or by the DCC method, followed by removal of ether groups with hydrogen chloride in chloroform, gave compounds (347) and (348).[508] The presence of the trityl group at the 2′- or 3′-position appears necessary for successful oxidation, since 5′-*O*-tritylthymidine was completely degraded by the above reagents.

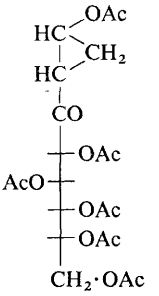
The following compounds have also been oxidised to the corresponding keto-derivatives with DMSO-based reagents: phenyl 4,6-*O*-benzylidene-2-deoxy-α-D-*arabino*-hexopyranoside,[509] methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*xylo*-hexopyranoside,[509] 1,2:3,4-di-*O*-isopropylidene-L-rhamnitol,[303] methyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside,[303] 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose,[303] 1,2-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-D-glucofuranose (to give a 3,5-diketo-compound) and its 3-deoxy-analogue[510] (the latter compound was described in the original paper as a D-galactofuranose, though the formula shown was that of the compound here named), and methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside.[303] For the last compound the use of reagents based on DCC was found to be better than DMSO–acetic anhydride.[303]

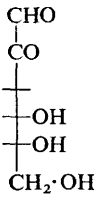
1,2:4,5,6-Penta-*O*-acetyl-D-*ribo*-hex-3-ulose has been prepared by standard procedures from 3-*O*-benzyl-D-glucitol.[511] Derivatives of 1,2:3,4-di-*O*-isopropylidene-D-*galacto*-hept-6-ulose (349) have been described.[512] The key reaction in their synthesis was that of diazomethane with 1,2:3,4-di-*O*-isopropylidene-D-galacturonyl chloride. Vinyl acetate reacted with diazoketone derivatives such as (350) in the presence of cupric ions, presumably *via* a carbene intermediate to give the chain-extended derivative (351) which contained a cyclopropyl ring. The reaction was also carried out on the D-*galacto*-, and D-*arabino*-analogues of (350).[513]

[508] A. F. Cook and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1967, **89**, 2697.
[509] K. Antonakis and F. Leclercq, *Compt. rend.*, 1967, *C*, 265, 1004.
[510] K. Antonakis, F. Leclercq, and M.-J. Arvor, *Compt. rend.*, 1967, *C***264**, 524.
[511] A. Sera and R. Goto, *J. Chem. Soc. Japan*, 1967, **88**, 790.
[512] S. David and M.-O. Popot, *Carbohydrate Res.*, 1967, **5**, 234.
[513] Yu. A. Zhdanov, V. I. Kornilov, and D. G. Mostovtseva, *Zhur. obshchei Khim.*, 1967, **37**, 2357.


(349)


(350)

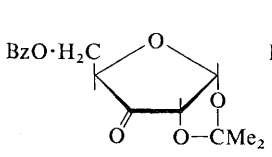

(351)

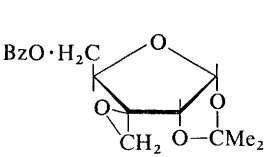

(352)

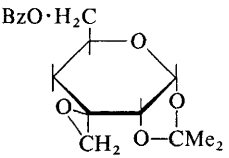
It has been shown that aldulose derivatives derived from compounds containing a free secondary hydroxy-group can be produced by oxidation with silver(II) picolinate (see p. 176).

3-Deoxyglucosone (3-deoxy-D-*erythro*-hex-2-ulose) (352) has been found to be biologically inactive [514] and is consequently not 'retine' as had been suspected. The true structure of the latter compound, which has been detected in tissue extracts and which inhibits malignant growth, therefore remains in doubt. 6-Deoxy-D-*arabino*-hexofuranos-5-ulose (329), the carbohydrate component of hygromycin A, has been synthesised.[485]

The ring-expansion reaction that occurs during treatment of cyclic keto-derivatives with diazomethane has been studied with 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuran-3-ulose (353).[515] Compounds (354) and (355) were obtained crystalline and in high yield, the proportions


(353)


(354)

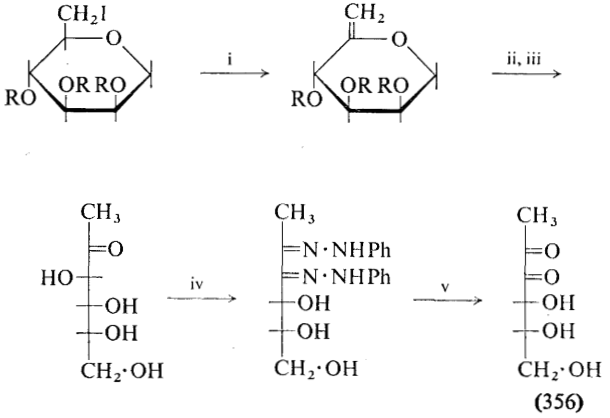

(355)

depending on the solvent used; with ether (354) was obtained in 70% yield, whereas with methanol–ether (354) and (355) were isolated in 25 and 65% yield respectively. The structures of the products were based on chemical analysis, and on lithium hydride reduction followed by periodate oxidation. Both products also gave rise to 3,6-anhydro-derivatives.

Aldulose derivatives have been used as intermediates in the synthesis of amino-sugars [367] and branched-chain sugars,[497, 499–501] and in the synthesis of epimers of the hydroxy-compounds from which they were derived (see Section 22).

[514] A. Szent-Györgyi, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 1642.
[515] S. Nahar, W. G. Overend, and N. R. Williams, *Chem. and Ind.*, 1967, 2114.

An authentic synthesis of 1-deoxy-D-*erythro*-hexo-2,3-diulose (356) has been achieved as shown in Scheme 37;[516] compound (356) is a postulated intermediate in the formation of saccharinic acids.



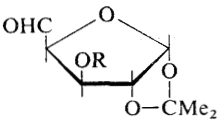
(356)

Reagents: i, AgF–py; ii, MeOH–MeONa; iii, aq. AcOH;
iv, $PhNH{\cdot}NH_2$, HCl–aq. NaOAc; v, HNO_2.

R = Bz.

Scheme 37

Methods have been investigated for the synthesis of asymmetric benzylic centres using carbohydrates as substrates.[517] Phenylmagnesium bromide reacted with 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (357) in ether to give the α-D-*gluco*- and β-L-*ido*-products in a



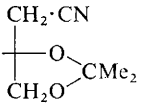
(357) R = CH_2Ph
(358) R = H

ratio of 1 : 14·4. In tetrahydrofuran the ratio was 1 : 3·3. When the C-3 —OH compound (358) was used the product ratios again changed. In ether, the *gluco* : *ido* ratio was 1 : 2·3, and in tetrahydrofuran it was 1 : 1, showing that ether was the more stereoselective solvent.

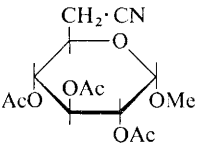
[516] A. Ishizu, B. Lindberg, and O. Theander, *Carbohydrate Res.*, 1967, **5**, 329.
[517] T. D. Inch, *Carbohydrate Res.*, 1967, **5**, 45.

17
Sugar Acids and Lactones

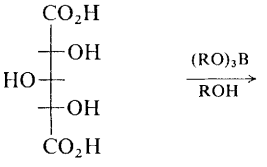
A general procedure has been developed for the replacement of a primary hydroxy-group in a carbohydrate by a nitrile group in a one-step reaction involving chlorodeoxy-intermediates.[518] This offers a means for preparing a wide variety of carboxylic acid derivatives. The alcohol was heated in carbon tetrachloride and triphenylphosphine successively with DMSO and sodium cyanide to give the nitrile directly. (The use of triphenylphosphine and carbon tetrachloride for the conversion of free sugars to glycosyl halides has been mentioned.[337]) While DMSO and carbon tetrachloride were found to be essential for the success of the reaction, the triphenylphosphine could be replaced by other phosphines. Water had to be excluded and it was advantageous to remove the chloroform formed during the first phase of the reaction before the sodium cyanide was added. By this method 1,2-*O*-isopropylideneglycerol and methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside were converted to the products (359) and (360) respectively.



(359)



(360)



(361)

(362)

A generally applicable esterifying reaction is suggested from the finding that trialkyl borates underwent transesterification with hydroxy-acids to give products which solvolyse to acyl esters. The alcoholic groups were also

[518] D. Brett, I. M. Downie, and J. B. Lee, *J. Org. Chem.*, 1967, **32**, 855.

converted to borate esters but these on solvolysis regenerated the free alcohols. The procedure was illustrated by conversion of *xylo*-saccharic acid (361) to its diester (362).[519]

Aldonic Acids

Silver picolinate in solvents such as water, DMSO, and DMSO–diglyme has been shown to oxidise secondary hydroxy-groups on carbohydrates to carbonyl groups, and has been employed to prepare lactones and thence aldonic acids. 2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose, for example, was converted to the corresponding γ-lactone derivative.[520] Platinum-catalysed oxidations of aldoses to aldono-δ-lactones have been reported previously but have now been applied to D-mannose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-glucuronic acid, D-galacturonic acid, D-galactose, L-arabinose, and L-fucose. Only the first three afforded crystalline products, but, in each case, solutions of the products contained compounds which acted as more potent glycosidase inhibitors than did the corresponding γ-lactones. It was tentatively postulated that glycosidase inhibition by appropriate lactones resulted specifically from the influence of the six-membered form.[521] More particularly, conditions have been described for the electrolytic oxidation of lactose and the isolation of calcium lactonate. Some degradation accompanied the reaction and calcium oxalate, calcium gluconate, and calcium D-*xylo*-5-hexulosonate were isolated.[522]

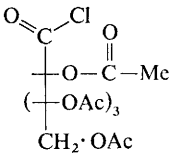
The reduction of lactones to free sugars also continues to attract attention; chemical methods using di-isopinocamphenyl borane[337] and bis-(3-methyl-2-butyl)borane (see p. 145) have been referred to. Electrolytic reduction has been applied commercially, and the factors influencing the preparation of D-ribose from the aldonolactone have been investigated. Ammonium salts were found to be preferable to alkali-metal salts as electrolytes, and boric acid was partially effective in preventing further reduction of the product to ribitol.[523] In other work by the same authors the sulphates of twenty-eight amines were assessed as electrolytes; the yields of D-ribose varied with the base and were found to be quantitative when hexylamine sulphate was used.[524] A polarographic study of five aldonolactones has been reported and the effects of boric acid on the reduction half-wave potentials have been considered.[525]

Several reactions of aldonic acids have been studied. The Ruff degradations of the calcium salts, for example, of D-gluconic and lactonic acids were examined in detail and conditions favourable for the formation of

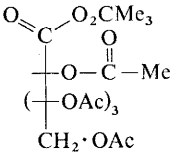
[519] V. V. Gertsev, *Zhur. obshchei Khim.*, 1967, **37**, 1481.
[520] J. B. Lee and T. G. Clarke, *Tetrahedron Letters*, 1967, 415.
[521] J. Conchie, A. J. Hay, I. Strachan, and G. A. Levvy, *Biochem. J.*, 1967, **102**, 929.
[522] M. L. S. Gupta, N. Bhattacharya, and U. P. Basu, *Indian J. Technol.*, 1967, **5**, 152.
[523] M. Matsumoto and M. Miyazaki, *J. Pharm. Soc. Japan*, 1967, **87**, 627.
[524] M. Matsumoto and M. Miyazaki, *J. Pharm. Soc. Japan*, 1967, **87**, 991.
[525] M. Matsumoto and R. Murata, *J. Pharm. Soc. Japan*, 1967, **87**, 101.

complexes between ferric ions, acids, and hydrogen peroxide which are believed to be required for this specific degradation were established.[526] With gluconate the complex was stable above pH 4 and dominant above pH 7. With lower pH the specificity of the oxidative decarboxylation was destroyed and non-specific reactions occurred.

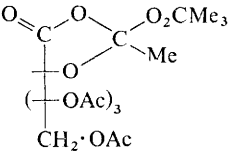
An alternative degradation has been reported in a continuation of the glycosyl peroxide work discussed on p. 25. Penta-*O*-acetyl-D-gluconyl chloride (363) with t-butyl hydroperoxide in the presence of a base such as pyridine gave t-butyl 2,3,4,5,6-penta-*O*-acetyl-D-peroxygluconate (364),



(363)



(364)



(365)

whereas without base the isomer (365) was formed. Decomposition of (364) or (365) (the former requiring more drastic treatment) with methanolic sodium methoxide gave D-arabinose. Similar isomeric esters were formed from tetra-*O*-acetylmucosyl chloride, but methoxide treatment caused only deacetylation.[527]

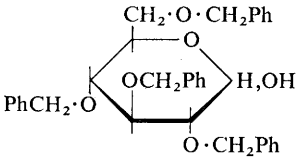
Normal aldonic acid esters may be synthesised using alcohols and acid catalysts but lactone formation can be a favoured competing reaction. However, when the hydroxy-groups were initially protected by acylation, esters were obtained without difficulty.[528] An alternative procedure which used transesterification from trialkylborates should be of interest in this respect.[519]

Aldono-γ-lactones are converted to hydrazides on treatment with hydrazine. The rates of the reactions with substituted phenylhydrazines have been measured, and it has consequently been shown that the reaction may be used for the separation of aldonolactones. Somewhat surprisingly, C-2, C-3-*cis*-compounds reacted less readily than did *trans*-isomers, but this is in keeping with relative rates of hydrolysis of aldono-γ-lactones.[529]

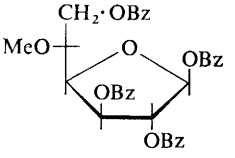
Kuzuhara and Fletcher[530] have used lactones in ingenious syntheses of 5-substituted aldoses and have thus provided a means for locking sugars in the furanosyl-ring form. Their method depended on the oxidation of a pyranose-ring compound, substituted at all positions except the anomeric centre, to the aldono-δ-lactone, opening the ring by converting the lactone into the appropriate amide, substituting the liberated 5-position, removing

[526] B. Larsen and O. Smidsrod, *Acta Chem. Scand.*, 1967, **21**, 552.
[527] M. Schulz and P. Berlin, *Angew. Chem. Internat. Edn.*, 1967, **6**, 950.
[528] W. J. Humphlett, *Carbohydrate Res.*, 1967, **4**, 157.
[529] H.-H. Stroh and D. Henning, *Chem. Ber.*, 1967, **100**, 388.
[530] H. Kuzuhara and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 2531.

the protecting groups and reducing back to the free sugar. The example cited described the synthesis of 1,2,3,6-tetra-*O*-benzoyl-5-*O*-methyl-β-D-glucofuranose (367) from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (366).



(366)

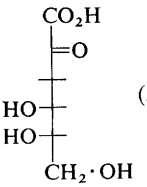


(367)

Oxidation was effected with DMSO and acetic anhydride (several other reagents failed), treatment with dimethylamine gave an amide which was methylated at C-5, and the benzyl groups were removed to give 5-*O*-methyl-D-glucono-γ-lactone. Reduction, after benzoylation, was brought about with bis-(3-methyl-2-butyl)borane. Alternatively, oxidation at the unprotected 5-position afforded a means for obtaining 5-keto-derivatives of aldonic acids, and also for causing specific inversions at that site, since reduction of the ketonic group can give rise to appreciable amounts of the 5-epimer.[531]

In related fashion, the furanosyl-ring form of lactones has been utilised in the synthesis of glycofuranosyl halides and thence furanosylnucleosides.[337]

D-Gluconic acid has been shown to be the major water-soluble constituent of the defensive secretion of *Eurycotis decipiens*, where it is present in



(368)

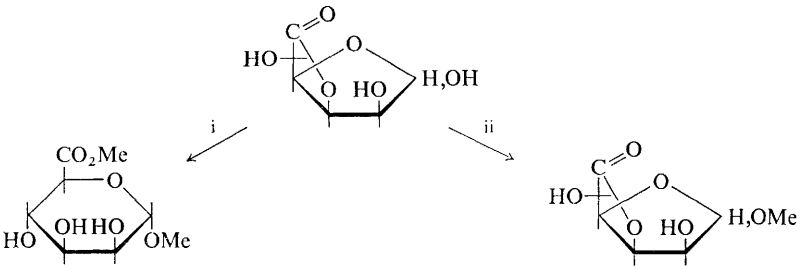
unusually high concentration.[532] The modified gluconic acid (368) was produced in good yield on degradation of 3-deoxy-D-*xylo*-hexose by *Pseudomonas putida*.[533]

Uronic Acids

The synthesis of methyl D-galacturonate has been studied with particular reference to the preparation of the ^{14}C-labelled methyl ester for use in biosynthetic work. Two routes were developed: from D-galactose by the platinium-catalysed oxidation of benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside, and from 1,2:3,4-di-*O*-benzylidene-D-galacturonic acid.[534]

[531] H. Kuzuhara and H. G. Fletcher, jun., *J. Org. Chem.*, 1967, **32**, 2535.
[532] G. P. Dateo and L. M. Roth, *Science*, 1967, **155**, 88.
[533] H. W. Schiwara and G. F. Domagk, *Z. physiol. Chem.*, 1967, **348**, 385.
[534] R. H. Shah and F. Loewus, *Carbohydrate Res.*, 1967, **4**, 401.

The methanolysis products from mannurono-γ-lactone have been shown to be dependent on the catalyst used, as shown in Scheme 38.[535]



Reagents: i, MeOH–HCl; ii, MeOH–Dowex 50.

Scheme 38

Gas chromatography may be applied with profit to the analysis of uronic acid-containing materials. Multiple peaks were observed on the examination of TMS derivatives derived from uronic acids themselves, their lactones, their methyl esters, and their methyl ester methyl glycosides. In some cases the peaks were related to particular isomers and the isomer ratios were recorded.[536] Other aspects of the analysis of uronic acids are referred to on p. 197.

Acid hydrolysis studies on uronosides and base-catalysed cleavage of 4-linked pseudoaldobiouronic acids have been discussed elsewhere.[113–117] Mixtures of aldobiouronic acids have been separated by ion-exchange chromatography using sodium tetraborate as eluent.[537]

Saccharinic Acids

1-Deoxy-D-*erythro*-hex-2,3-diulose, the postulated intermediate in the formation of D-glucosaccharinic acid, has been synthesised chemically and it rearranged, as expected, on treatment with alkali.[516] Furthermore, α-D-glucoisosaccharinic acid has been shown to be 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid by *X*-ray crystallographic analysis on the calcium and strontium salts.[538]

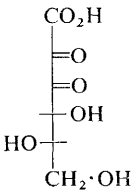
A thorough investigation has been carried out on the saccharinic acids produced by the action of calcium and sodium hydroxide on D-xylose and D-fructose; the products were converted into their lactones and fractionated both on cellulose columns and by gas chromatography of the TMS derivatives. From D-xylose, fourteen lactones were detected, and all but one were characterised by their chromatographic properties. The

[535] H. W. H. Schmidt, *Tetrahedron Letters*, 1967, 235.
[536] O. Raunhardt, H. W. H. Schmidt, and H. Neukom, *Helv. Chim. Acta*, 1967, **50**, 1267.
[537] O. Samuelson and L. Wictorin, *Carbohydrate Res.*, 1967, **4**, 139.
[538] M. von Glehn, P. Kierkegaard, R. Norrestam, O. Rönnquist, and P.-E. Werner, *Chem. Comm.*, 1967, 291.

main acids were 3-deoxy-*erythro*- and *threo*-pentonic acids but the previously unknown 2-*C*-methyl-D-erythronic and -threonic acids were also isolated and characterised. Four, five, and six carbon acids were detected so that the degradation involved fragmentations and recombinations in addition to isomerisations. From D-fructose essentially the same products were obtained but in different proportions and 2-*C*-methyl-D-ribonic acid predominated. The paper also described brief examinations of the degradation of L-arabinose.[539]

Ascorbic Acid

Some slight developments have been described in the chemistry of ascorbic acid. Compounds considered to be the 6-stearate and 6-benzoate have been reported from the condensation of the respective acids in the unusual solvent 50% sulphuric acid–nitromethane.[540] Three papers by Japanese workers[541] have described the degradation and nonenzymic browning reaction. With acid, furfural was formed *via* a 3-deoxy-osone, and dehydroascorbic acid gave furfural, ethylglyoxal, 3-deoxy-2-keto-pentono-γ-lactone, and xylosone. Other workers have carried out a kinetic investigation of the hydrolysis of this latter compound to L-*threo*-hex-2,3-dilusonic acid (369).[542]



(369)

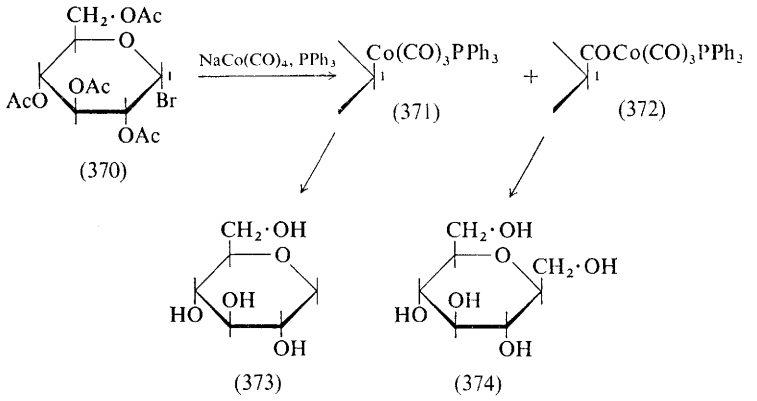
The first of a series of investigations on the ionic equilibria existing in solutions of ascorbic acid and salts has been reported.[543]

[539] A. Ishizu, B. Lindberg, and O. Theander, *Acta Chem. Scand.*, 1967, **21**, 424.
[540] J. Kulesza, J. Gora, and J. Krygier, *Zeszyty Nauk. Politech. lodz.* (*Chem.*), 1967, **12**, 29.
[541] T. Kurata and Y. Sakurai, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 101 (with H. Wakabayashi), 170 and 177.
[542] G. Von Foerster, W. Weis, and H. Staudinger, *Z. physiol. Chem.*, 1967, **348**, 236.
[543] O. Wahlberg and P. Ulmgren, *Acta Chem. Scand.*, 1967, **21**, 2759.

18
Inorganic Derivatives

Carbon-bonded Compounds

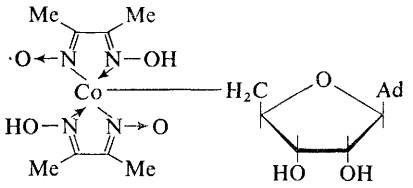
Cobalt derivatives of carbohydrates have been formed by displacements of readily ionisable groups. Tetra-*O*-acetyl-α-D-glucosyl bromide (370) on treatment with the cobalt tetracarbonyl anion gave two products that were isolated as their triphenylphosphine complexes, (371) and (372), and which



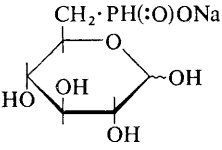
on deacetylation followed by sodium borohydride reduction gave the anhydroalditols (373) and (374).[544] However, less reactive halides than those of glycosyl compounds may be displaced since treatment of chloropyridinebis(dimethylglyoxime)cobalt with sodium borohydride gave a hydride which, with 6-*N*-acetyl-5′-bromo-5′-deoxyadenosine 2′,3′-phenylboronate, afforded the organocobalt derivative of 5′-deoxy-adenosine[545] with the probable structure (375). In related work the partial chemical synthesis of the vitamin B_{12} coenzyme adenosyl-B_{12} has been improved by using 2′,3′-*p*-dimethylaminobenzylidene-5′-*O*-toluene-*p*-sulphonyladenosine. This suffered sulphonyloxy-group displacement on reaction with the reduced form of the vitamin B_{128} and a cobalt —C-5′ bond was formed.

[544] A. Rosenthal and H. J. Koch, *Tetrahedron Letters*, 1967, 871.
[545] A. M. Yurkevich, I. P. Rudakova, and N. A. Preobrazhenskii, *Khim. prirod. Soedinenii*, 1967, **3**, 48.

The coenzyme was then obtained under very mild acidic conditions; use of the isopropylidene blocking group resulted in secondary reactions and reduction in yields.[546]

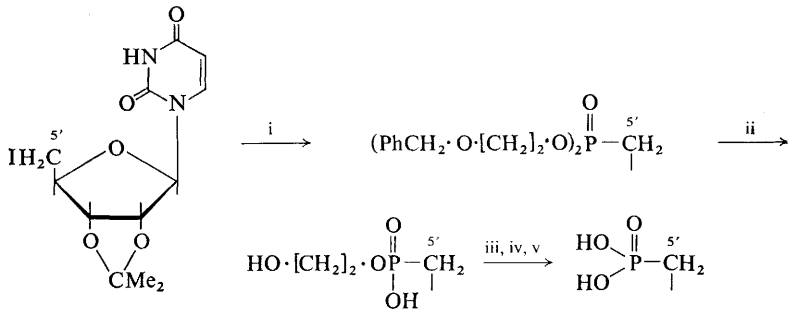


(375)



(376)

An example of a compound with a carbon to phosphorus bond was encountered on heating 1,6-anhydro-D-glucose with hypophosphorous acid and sodium hypophosphite. The resulting glycosylphosphinic acid (376), which was purified on cellulose, was stable to acid and on reduction gave the glucitol derivative.[547] A phosphonic acid derivative of a nucleoside has been prepared [548] as shown in Scheme 39.



Reagents: i, $(PhCH_2 \cdot O \cdot [CH_2]_2 \cdot O)_3P$; ii, H_2–Pd–AcOH; iii, $(Ph_3PMe)^+I^-$; iv, Et_3N; v, HO^-.

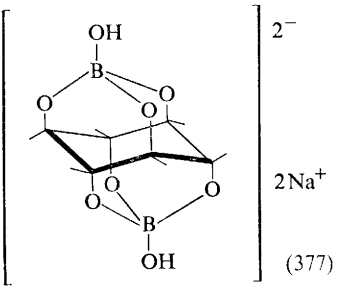
Scheme 39

Oxygen-bonded Compounds

Several reports on the interaction of polyhydroxy-compounds with inorganic acids have appeared. The borate complex (377) previously obtained on sodium borohydride reduction of the 'all equatorial' inosose has now been shown to be readily accessible by mixing aqueous solutions of *scyllo*-inositol and borax. The structure was confirmed by the observation that the ring protons gave a singlet in the n.m.r. spectrum and were

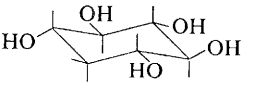
[546] R. R. Schmidt and F. M. Huennekens, *Arch. Biochem. Biophys.*, 1967, **118**, 253.
[547] N. K. Kochetkov, E. E. Nifantev, and I. P. Gudkova, *Zhur. obshchei Khim.*, 1967, **37**, 277.
[548] A. Holý, *Tetrahedron Letters*, 1967, 881.

therefore identical.[549] Equilibrium constants involved in the reaction were determined from the elevation of pH noted on addition of the inositol to borax solution, and a simple method based on ester exchange was described

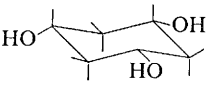


(377)

for the regeneration of the inositol from the complex.[549] The same authors determined the formation constants for the tridentate borate complexes of the cyclohexane pentaol (378) and triol (379).[550]



(378)



(379)

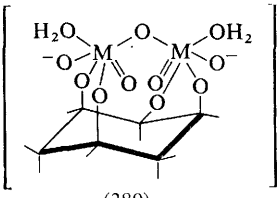
The interaction between nucleosides and boric acid has been studied by potentiometric methods in order to throw light on the known effect of the reagent on nucleic acid synthesis. Weak 2 : 1, nucleoside–boric acid complexes were postulated and chelation constants were recorded. It was concluded that borate could interfere in RNA synthesis by interacting with the 2′,3′-diol group of the nucleotides.[551]

A molybdate complex has been isolated from *epi*-inositol as its ammonium and sodium salts; likewise, tungstate gave a complex which was isolated as its sodium salt. Chemical analysis and molecular weight determinations showed that each complex contained cyclitol, molybdate (or tungstate), and sodium (or ammonium) in the ratios 1 : 2 : 2, and n.m.r. spectroscopy indicated the structure (380).[550] Examination of the electrophoretic mobilities of a large number of cyclitols revealed that those having *cis*-hydroxy-groups in the 1, 2, 3, and 5 relationship complexed most strongly. Complexes were also obtained from those compounds having *cis*-hydroxy-groups at positions 1, 2, 3, and 4, but with less ease.[550]

Gallium and indium complex with gluconic acid in a process which has been studied by a distribution technique involving water and chloroform

[549] T. Posternak, E. A. C. Lucken, and A. Szente, *Helv. Chim. Acta*, 1967, **50**, 326.
[550] T. Posternak, D. Janjic, E. A. C. Lucken, and A. Szente, *Helv. Chim. Acta*, 1967, **50**, 1027.
[551] U. Weser, *Z. Naturforsch.*, 1967, *B*, **22**, 457.

at pH 10–11, and 1 : 1 complexes have been isolated,[552] and the complexes formed by lanthanum and cerium have been investigated by electrophoretic methods.[553] The interaction between germanic acid and D-glucaric acid has been studied by potentiometric titration and spectrophotometrically.[554]



(380)

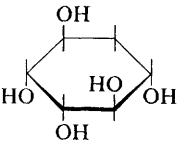
Reference is made to other complexes on p. 157.

[552] C. G. Macorovici, E. Perte, and E. Motiu, *Rev. Roumaine Chim.*, 1967, **12**, 975.
[553] G. Marcu and G. Murgu, *Rev. Roumaine Chim.*, 1967, **12**, 957.
[554] C. G. Macarovici and M. Volusniuc-Birou, *Rev. Roumaine Chim.*, 1967, **12**, 163.

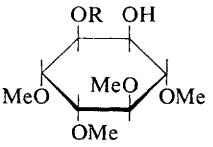
19
Cyclitols

The chemistry of cyclitols, including the synthesis of inosamines, has been reviewed,[555a] as have their occurrence, metabolism, and biosynthesis in a review dated 1966, that appeared in 1967.[555b]

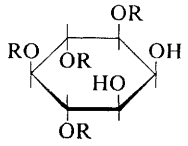
An inosityl ester of indole-3-acetic acid, isolated from corn kernels, has been tentatively identified as 2-*O*-(indole-3-acetyl)-*myo*-inositol; its biological role is not yet understood.[556] (−)*proto*-Quercitol (381) has been synthesised from (−)-inositol.[557] Use of the mono-*O*-isopropylidene



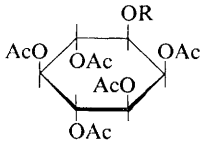
(381)



(382) R = H
(383) R = Me



(384) R = CH_2Ph



(385) R = CH_2Ph
(386) R = H

derivative of the latter compound gave a tetra-*O*-methyl derivative (382); this was selectively esterified at the C-2-equatorial hydroxy-group with toluene-*p*-sulphonyl chloride, then methylated further to give, after alkaline treatment, the penta-*O*-methyl ether (383). Oxidation with ruthenium dioxide–sodium periodate gave the corresponding keto-compound, which was treated with 1,2-ethanedithiol, the product desulphurised, and the ether groups cleaved to give the required compound (381).

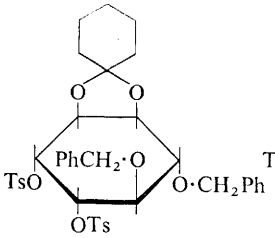
[555a] G. Kimura and E. Noda, *Yuki Gosei Kagaku Kyokai Shi*, 1967, **25**, 764.
[555b] H. Kindl and O. Hoffman-Ostenhof, *Prog. Chem. Org. Nat. Prod.*, 1966, **24**, 149.
[556] P. B. Nicholls, *Planta*, 1967, **72**, 258.
[557] G. E. McCasland, M. O. Naumann, and L. J. Durham, *Carbohydrate Res.*, 1967, **4**, 516.

Several papers on cyclitol chemistry have come from Angyal's laboratory. The acetolysis of benzyl ethers of *myo*-inositol has been studied;[558] the reaction was a selective one and the rate of the reaction varied with the nature and the configuration of neighbouring groups. Thus, acetolysis of the tetra-*O*-benzyl ether (384) at 0° gave the penta-*O*-acetyl-mono-*O*-benzyl derivative (385), which, on hydrogenolysis, gave the sought-after 1,2,4,5,6-penta-*O*-acetyl-*myo*-inositol (386). At higher temperatures hexa-*O*-acetyl-*myo*-inositol was the product from the acetolysis of (384). These results and others from partial acetolyses suggested that the reaction was subject to steric influences, that *cis*-groups presented a greater hindrance than did *trans* ones, and that an acetoxy-group hindred more than a benzyloxy-group.

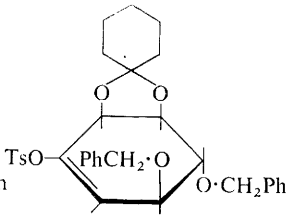
In attempts to develop syntheses of rare compounds, several mono-*O*-toluene-*p*-sulphonyl derivatives of inositols have been subjected to solvolysis in boiling 95% acetic acid containing sodium acetate.[559] No consistent reaction was observed; in some cases displacement occurred with inversion, in others, appreciable retention was observed. The inversion was satisfactorily utilised for the synthesis of *allo*- and *muco*-inositols, and their methyl ethers.

With the same objective, toluene-*p*-sulphonyl esters of (+)- and (−)-inositol were treated with boiling DMF, both with and without sodium benzoate.[560] Specific displacements generally occurred if a neighbouring *trans*-acetoxy-group was present, when the reaction was postulated as occurring *via* a cyclic acetoxonium ion, which hydrolysed to give the *cis*-product. Reactions based on this approach were recommended for the synthesis of *allo*- and *muco*-inositol. In a few cases such displacements did not occur, presumably because the toluene-*p*-sulphonyloxy-group and the acetoxy-group could not assume the required diaxial conformation. When vicinal *cis*-toluene-*p*-sulphonyloxy-groups were present, treatment in the presence of sodium benzoate caused elimination; for example, (387) gave (388). Work on *cis*-1,2-ditoluene-*p*-sulphonyloxycyclohexane (389) gave the products (390), (391), and (392) in yields of 8, 21, and 53% respectively, showing that the presence of the vicinal *cis*-diester group is not the sole requirement for the elimination reaction. In systems in which the ester group had a neighbouring *trans*-hydroxy-group, displacement occurred *via* the corresponding epoxide, which opened to give the products, but not in the proportions expected. This was explained by invoking stabilisation by intramolecular hydrogen-bonding of the developing anion, subsequent upon attack at the three-membered ring. Such stabilisation did not occur in protic solvents because of competitive solvation.

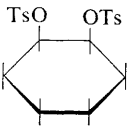
[558] S. J. Angyal, M. H. Randall, and M. E. Tate, *J. Chem. Soc.* (*C*), 1967, 919.
[559] S. J. Angyal, V. J. Bender, P. T. Gilham, R. M. Hoskinson, and M. E. Pitman, *Austral. J. Chem.*, 1967, **20**, 2109.
[560] S. J. Angyal and T. S. Stewart, *Austral. J. Chem.*, 1967, **20**, 2117.



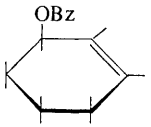
(387)



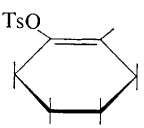
(388)



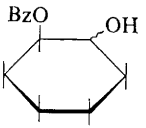
(389)



(390)

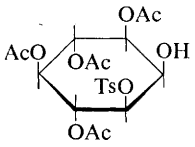


(391)

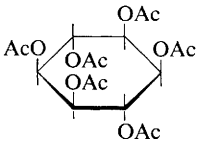


(392)

A third new synthesis of *muco*-inositol has been described.[561] Solvolysis of 1,4,5,6-tetra-*O*-acetyl-3-*O*-toluene-*p*-sulphonyl-*myo*-inositol (393), or the corresponding 3-*O*-methanesulphonyl derivative, in boiling 90% aqueous 2-methoxyethanol containing sodium acetate, gave hexa-*O*-acetyl-*muco*-inositol (394) which could be deacetylated to the parent compound. The course of this reaction was presumed to proceed *via* an acetoxonium ion that was then opened in a *trans*-diaxial manner by acetate ion.



(393)



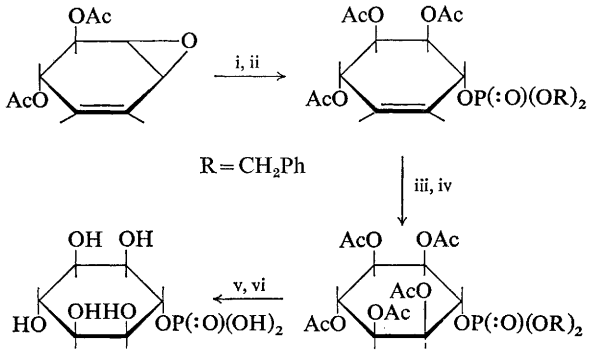
(394)

Exchange of (−)-inositol with tritiated water on platinum oxide led to tritiation mainly at C-1, with partial inversion of configuration (to give *myo*-inositol). Possible mechanisms were discussed and the process was compared with the Wilzbach technique.[562, 563]

Reaction of *scyllo*-inositol with 1,1-dimethoxycylohexane and toluene-*p*-sulphonic acid in DMF under conditions in which the methanol was removed as it was formed gave[222] the 1,2:3,4:5,6-tri-*O*-cyclohexylidene derivative.

[561] T. Suami, F. W. Lichtenthaler, and S. Ogawa, *Bull. Chem. Soc. Japan*, 1967, **40**, 1488.
[562] S. J. Angyal, C. M. Fernandez, and J. L. Garnett, *Austral. J. Chem.*, 1967, **20**, 2647.
[563] C. M. Fernandez and J. L. Garnett, *J. Labelled Compounds*, 1967, **3**, 155.

Several inositol monophosphates, isolated as their cyclohexylammonium salts, have been made using sequences of the type shown in Scheme 40.[564]



Reagents: i, $(RO)_2P(:O)OH$–$CHCl_3$; ii, Ac_2O–py; iii, $KMnO_4$; iv, Ac_2O–py; v, H_2–Pd–$BaSO_4$; vi, MeONa–MeOH.

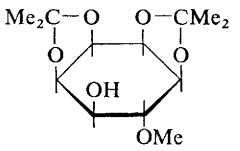
Scheme 40

Oxidation of *epi*-, *myo*-, *dextro*-, *laevo*-, *muco*-, or *scyllo*-inositol with DMSO and acetic anhydride in the presence of pyridine gave a good yield of penta-acetoxybenzene. It was suggested that the reaction proceeded *via* a diketo-inositol intermediate.[565] It has been reported that photolysis of oxygenated aqueous solutions of *myo*-inositol gave *myo*-inos-2-one with saccharic acids and formic acid.[566] It was concluded that whereas direct oxidation occurred preferentially at an axial hydroxy-group, ring-opening reactions to give saccharic acids and further degradation products took place more readily at *cis*-diequatorial diol sites. The acidic products formed were not completely characterised. Oxidation of a series of cyclohexane-1,2,3,4-tetrols by *Acetobacter suboxydans* has been shown to occur at axial hydroxy-groups [567] (cf. p. 176); *scyllo*-inositol was oxidised as shown on p. 176.

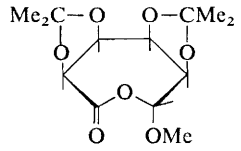
The hemiacetal lactone (396) has been prepared from 1,2:3,4-di-*O*-isopropylidene-6-*O*-methyl-*epi*-inositol (395) either by oxidation with active manganese dioxide, or with the Pfitzner–Moffatt reagent followed by a Baeyer-Villiger reaction.[568] The formation of the lactone (396) provides a route to hexoses from cyclitols. Reduction of (396) with lithium aluminium hydride gave 2,3:4,5-di-*O*-isopropylidene-DL-allitol, whilst treatment with methanolic sulphuric acid, followed by acetylation, gave a mixture of the alluronic acid ester derivatives (397) and (398), which by

[564] N. Kurihara, H. Shibata, H. Saeki, and M. Nakajima, *Annalen*, 1967, **701**, 225.
[565] A. J. Fatiadi, *Chem. Comm.*, 1967, 441.
[566] W. J. Criddle, B. Jones, and E. Ward, *Chem. and Ind.*, 1967, 1833.
[567] P. Barbezat, D. Reymond, and T. Posternak, *Helv. Chem. Acta*, 1967, **50**, 1811.
[568] H. Fukami, H.-S. Koh, T. Sakata, and M. Nakajima, *Tetrahedron Letters*, 1967, 4771.

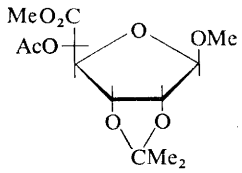
standard sequences could be converted into methyl 2,3,5,6-tetra-*O*-acetyl-β-DL-allofuranoside.



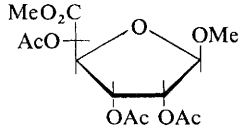
(395)



(396)

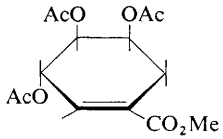


(397)

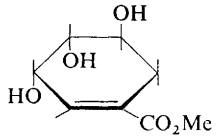


(398)

By standard reactions, cyclohexadienecarboxylic acid has been converted into the 4-epishikimic acid derivative (399) which, on treatment with liquid hydrogen fluoride, gave shikimic acid methyl ester (400) in 80% yield,[569] providing, therefore, a simple route to the acid.



(399)

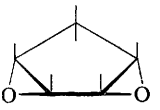


(400)

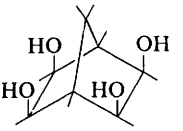
A detailed study[570] has been made of directive effects in the reactions of cyclopentanetetrol derivatives. Epoxide ring-openings have been shown to occur at the position furthest from a vicinal electronegative substituent. The *cis*-diepoxide (401), however, showed no stereoselectivity in its ring-opening reactions. In the acid hydrolysis of mono-epoxido-mono-*O*-isopropylidene derivatives of cyclopentanetetrols, it was shown that the acetal group was first removed, with subsequent ring-opening. The conformation of cyclopentanetetrols was discussed in terms of hydrogen-bonding and nonbonded interactions. *exo-exo*-2,3,5,6-Bicyclo[2,2,1]heptanetetrol (402) has been studied as a model compound for the reactions of cyclopentanetetrols.[571]

[569] R. Grewe and S. Kersten, *Chem. Ber.*, 1967, **100**, 2546.
[570] B. Tolbert, R. Steyn, J. A. Franks, jun., and H. Z. Sable, *Carbohydrate Res.*, 1967, **5**, 62.
[571] H. Z. Sable and H. Katchman, *Carbohydrate Res.*, 1967, **5**, 109.

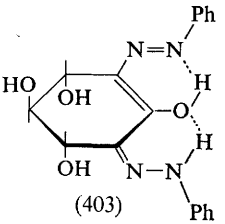
A study of the tautomerism exhibited by *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (403) and by related compounds has been made using spectroscopic techniques.[572] Borate [549] and molybdate [550] complexes of inositols have been investigated.



(401)



(402)

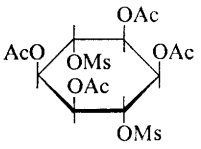


(403)

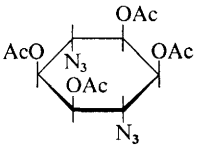
Amino-cyclitols

Much interest has been shown in the synthesis of streptamine, its 2-deoxy-analogue, and actinamine, all antibiotic components.

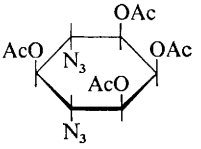
muco-Inositol has been converted into its useful 1,2,5,6-tetra-*O*-acetyl-3,6-di-*O*-methanesulphonyl derivative (404) *via* its di-*O*-isopropylidene derivative.[561] Treatment of (404) with sodium azide in aqueous 2-methoxyethanol gave 1,2,3,5-tetra-*O*-acetyl-4,6-diazido-4,6-dideoxy-*myo*-inositol (406) and 1,2,4,5-tetra-*O*-acetyl-3,6-diazido-3,6-dideoxy-*muco*-inositol (405).



(404)



(405)

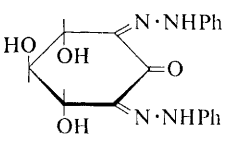


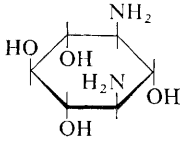
(406)

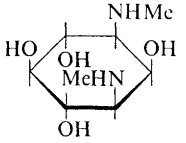
The formation of these products was explained *via* acetoxonium intermediates. Similarly,[561] 2,4,5,6-tetra-*O*-acetyl-1,3-di-*O*-toluene-*p*-sulphonyl-*myo*-inositol gave 2,3,5,6-tetra-*O*-acetyl-1,4-diazido-1,4-dideoxy-*muco*-inositol and 3,6-di-*O*-acetyl-1,2,4,5-tetra-*O*-methanesulphonyl-*muco*-inositol yielded the corresponding 1,2,4,5-tetra-azido-1,2,4,5-tetradeoxy-derivative. In all the above cases, the azido-deoxy-compounds were reduced and the products characterised as their peracetyl derivatives.

[572] A. J. Fatiadi and H. S. Isbell, *Carbohydrate Res.*, 1967, **5**, 302.

The tricarbonyl bisphenylhydrazone derivative (407) (cf. ref. 572) (prepared in three steps from *myo*-inositol) can be reduced with sodium amalgam in acetic acid to give streptamine (408) isolated as its hexa-acetate in 30% yield.[573, 574] Catalytic reduction of (407) in the presence of

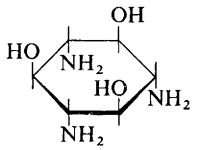

(407)

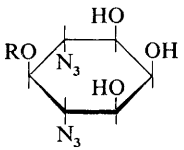

(408)

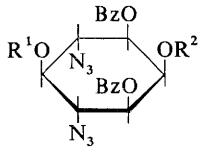

(409)

platinium occurred in an all-*cis* sense to give *myo*-inosa-1,3-diamine which on *N*-formylation and lithium aluminium hydride reduction gave actinamine (409).

By a series of standard conversions 1,2,3,5-tetra-*O*-acetyl-4,6-diazido-4,6-dideoxy-*myo*-inositol (406) has been converted into streptamine (408), actinamine (409), and *scyllo*-inosa-1,3,5-triamine (410), all isolated as hexa-acetyl derivatives.[575] The key reaction was the deacetylation of (406) with methanolic ammonia to give the mono-*O*-acetyl derivative (411), and the partial benzoylation of it, and of (412) [from acid hydrolysis of (406)] to give the di-*O*-benzoyl derivatives (413) and (414), respectively. In the case of (411) this was equatorial versus axial acylation and for (412), with *three*


(410)


(411) R = Ac
(412) R = H

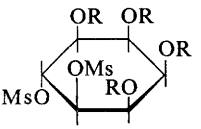

(413) R^1 = Ac, R^2 = H
(414) R^1 = H = R^2
(415) R^1 = Ac, R^2 = Ms

equatorial hydroxy-groups, the C-5-equatorial hydroxy-group must be hindered. The 2-*O*-methanesulphonyl derivative (415) when solvolysed in 2-methoxyethanol, containing sodium acetate, followed by acetylation, catalytic reduction, and *N*-acetylation gave hexa-acetylstreptamine. Treatment of (415) with sodium azide in the same solvent gave a triazido-derivative which was reduced and acetylated to *scyllo*-inosa-1,3,5-triamine (410) isolated as its hexa-acetyl derivative. The dihydroxy-derivative

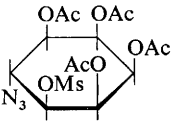
[573] F. W. Lichtenthaler, H. Leinert, and T. Suami, *Angew. Chem. Internat. Edn.*, 1967, **6**, 254.
[574] F. W. Lichtenthaler, H. Leinert, and T. Suami, *Chem. Ber.*, 1967, **100**, 2383.
[575] S. Ogawa, T. Abe, H. Sano, K. Kotera, and T. Suami, *Bull. Chem. Soc. Japan*, 1967, **40**, 2405.

(414) was converted into the corresponding di-*O*-methanesulphonyl compound which underwent S_N2 displacement with acetate ion to give a product that was reduced and *N*-acetylated to hexa-acetyl-*myo*-inosa-1,3-diamine; this was converted into the corresponding 2,4,5,6-tetra-*O*-acetyl-*NN*-diethoxycarbonyl derivative which, after lithium aluminium hydride reduction and acetylation, gave actinamine (409) as its hexa-acetyl derivative.

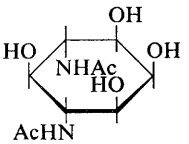
5,6-Di-*O*-methanesulphonyl-*epi*-inositol (416) (prepared *via* the di-*O*-isopropylidene derivative) with sodium azide in 2-methoxyethanol, followed by acetylation, gave 1,2,3,5-tetra-*O*-acetyl-4,6-diazido-4,6-dideoxy-*myo*-inositol (406) as the main product together with 1,2,3,4-tetra-*O*-



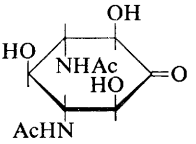
(416) R = H
(417) R = Ac



(418)



(419)

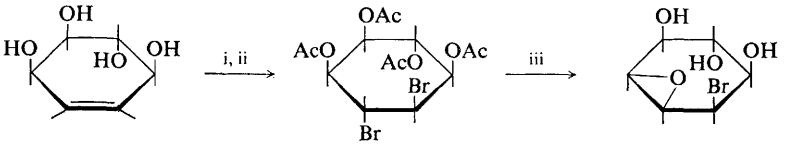


(420)

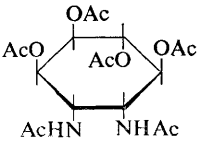
acetyl-6-azido-6-deoxy-5-*O*-methanesulphonyl-*epi*-inositol (418) in a ratio of 7 : 1.[576] With the tetra-*O*-acetyl derivative (417), (406) and (418) were produced in a ratio of 2·4 : 1. It was assumed that the displacement reaction on (416) occurred *via* epoxide intermediates, whereas those of (417) occurred *via* acetoxonium ions. Compound (406) was converted into (419) by standard reactions, and this was selectively oxidised by oxygen in the presence of platinum to (420) which could be reduced with sodium amalgam and then acetylated to give streptamine (408) as its hexa-acetyl derivative.

Another synthesis of streptamine (408) (as its hexa-acetyl derivative) has been developed, the critical steps of which are shown in Scheme 41.[577] Treatment of either (421) or (422) with sodium azide in DMF gave two azides, one of which was reduced and acetylated to give hexa-acetyl streptamine, the other on similar treatment gave hexa-acetyl-*rac*-inos-1,2-diamine (423).

[576] T. Suami and S. Ogawa, *Bull. Chem. Soc. Japan*, 1967, **40**, 1925.
[577] N. Kurihara, T. Kurokawa, and M. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 1166.



(421) (422)



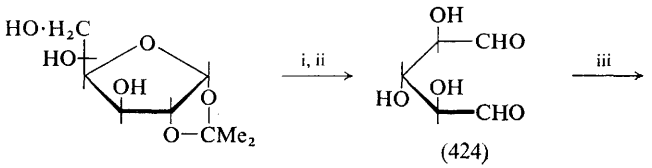
(423)

Reagents: i, Br_2–AcOH; ii, Ac_2O–py; iii, MeOH–MeONa.

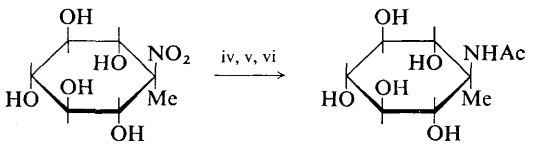
Scheme 41

6-*O*-Acetyl-1-*O*-toluene-*p*-sulphonyl-*epi*-inositol has been converted by azide displacement and standard reactions into hexa-acetyl-*epi*-inos-1-amine (again an acetoxonium intermediate was presumed) and 1,2-anhydro-*cis*-inositol gave hexa-acetyl-*epi*-inos-6-amine by opening with methanolic ammonia and acetylation.[576]

The *C*-methyl branched-chain inosamine derivative (425) has been synthesised using a nitroethane condensation on *xylo*-pentodialdose (424), as shown in Scheme 42; the configuration of the product was established by n.m.r. spectroscopy.[578]



(424)



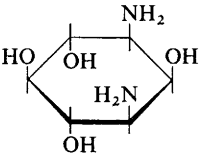
(425)

Reagents: i, IO_4^-; ii, H^+; iii, $EtNO_2$–MeO^-; iv, H_2–Pt; v, Ac_2O–NaOAc; vi, NH_3–MeOH.

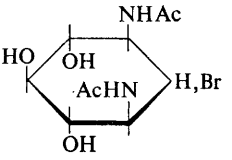
Scheme 42

[578] F. W. Lichtenthaler and H. K. Yahya, *Carbohydrate Res.*, 1967, **5**, 485.

myo-Inosa-1,3-diamine (426) dihydrochloride on treatment with acetyl bromide and acetic anhydride gave, after acetylation, compound (427), which catalytically reduced to penta-acetyl-2-deoxystreptamine (428).[579] The catalytic reduction of 4,6-dinitropyrogallol has been investigated as a



(426)



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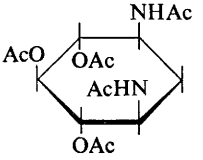


(428)

route to 2-deoxystreptamine;[580] none was found in the reduction mixture. The products isolated had, as would be expected from this type of reaction, either the all-*cis* or predominantly-*cis* configurations.

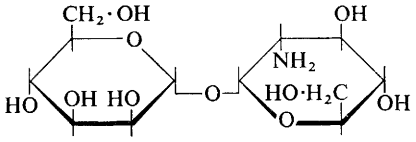
[579] T. Suami, S. Ogawa, and H. Sano, *Tetrahedron Letters*, 1967, 2671.
[580] H. H. Baer and R. J. Yu, *Tetrahedron Letters*, 1967, 807.

20
Antibiotics

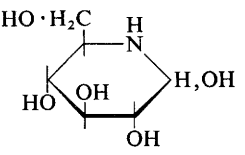
As in previous years there has been much activity in this area, particularly by Japanese workers. Many novel amino-sugars have been found as antibiotic components.

Antibiotics of current interest, including kasugamycin, moenomycin, lincomycin, and blasticidin S, have been reviewed,[581] as has the last-named substance, in particular.[582]

Acid hydrolysis of moenomycin (from *Streptomyces bambergiensis*) has been shown to yield 2-amino-2-deoxy- and 2-amino-2,6-dideoxy-D-glucose, as well as D-glucose and an, as yet, unidentified neutral sugar.[583] A new antibiotic (from *Streptomyces virginiae var* 4243-MT) has been shown to be α-D-mannopyranosyl 2-amino-2-deoxy-α-D-glucose (429), a simple nonreducing disaccharide.[584]



(429)



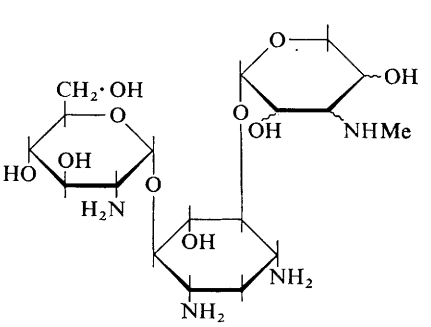
(430)

A further paper has appeared on nojirimycin, confirming that it is the first natural compound with a nitrogen atom in place of the ring oxygen, namely 5-amino-5-deoxy-D-glucose (430).[456] Gentamicin A has been shown to have the structure (431) in which one of the components is an, as yet, unidentified 3-methylamino-3-deoxypentopyranose (gentosamine).[353] A related branched-chain pentose, a 3-deoxy-4-*C*-methyl-3-methylamino-pentose (garosamine), has been isolated from gentamicin C, and has the partial structure (342) or its enantiomer.[352] Streptozotocin has been shown to be a 2-amino-2-deoxy-D-glucose derivative (432) with an *N*-nitroso-methylamide group.[355]

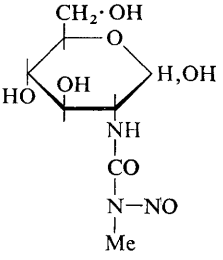
Full details have appeared on the structures of the methyl aldgarosides (A) and (B), isolated from methanolysis of aldgamycin. They are the

[581] S. A. Goulden, *Manuf. Chem.*, 1967, **38**, 36.
[582] N. Otake, *J. Agric. Chem. Soc. Japan*, 1967, **41**, R1.
[583] G. Huber, *Annalen*, 1967, **707**, 170.
[584] M. Uromoto, N. Otake, and H. Yonehara, *J. Antibiotics (Japan), Ser. A*, 1967, **20**, 236.

epimeric glycosides (343) and thus aldgarose is the first example both of a new class of naturally occurring branched-chain sugars and of a naturally occurring sugar carbonate.[502]

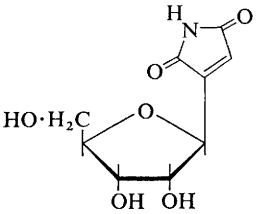


(431)

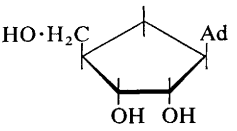


(432)

Work by two groups on showdomycin has proved it to be a *C*-nucleoside (433), namely, 3-(β-D-ribofuranosyl)maleimide.[585-587] Aristeromycin has been shown to be a close analogue of a nucleoside, namely the hydroxymethylcyclopentanediol derivative of adenine (434).[588] [It is interesting to note that the laboratory synthesis of (434) was reported late in 1966.]



(433)



(434)

Leucomycins A_3[589, 590] and A_4, A_5, A_6, A_7, A_8, and A_9[591] have been shown to contain mycaminose (3,6-dideoxy-3-dimethylamino-D-glucose) and different 4-*O*-acyl derivatives of mycarose (2,6-dideoxy-3-*C*-methyl-L-*ribo*-hexose), as the carbohydrate components: leucomycin A_3 contained

[585] K. R. Darnall, L. B. Townsend, and R. K. Robins, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 548.
[586] Y. Nakagawa, H. Kano, Y. Tsukuda, and H. Koyama, *Tetrahedron Letters*, 1967, 4105.
[587] Y. Tsukuda, Y. Nakagawa, H. Kano, T. Sato, M. Shiro, and H. Koyama, *Chem. Comm.*, 1967, 975.
[588] T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. Comm.*, 1967, 852.
[589] S. Omura, H. Ogura, and T. Hata, *Tetrahedron Letters*, 1967, 1267.
[590] S. Omura, M. Katagiri, H. Ogura, and T. Hata, *Chem. and Pharm. Bull. (Japan)*, 1967 **15**, 1529.
[591] S. Omura, M. Katagiri, and T. Hata, *J. Antibiotics (Japan), Ser. A.*, 1967, **20**, 234.

the isovaleryl ester, A_4 and A_5 the butyl ester, A_6 and A_7 the propionyl ester, and A_8 and A_9 the acetyl ester. Full details [592] have appeared on the structural studies on chromomycins A_2, A_3, and A_4, previously reported in a series of Communications. Five 2,6-dideoxy-hexoses (see Table 3) were found to be present, linked to the aglycone, chromomycinone (CHR) in different combinations as shown in Table 4.

Table 3

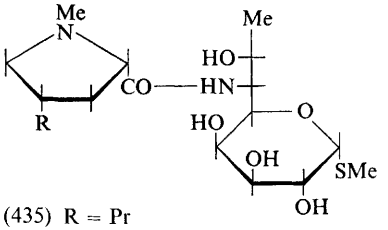
Chromose A	2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexopyranose
Chromose B	2,6-dideoxy-3-*C*-methyl-4-*O*-acetyl-L-*arabino*-hexopyranose
Chromose B′	2,6-dideoxy-3-*C*-methyl-4-*O*-isobutyryl-L-*arabino*-hexopyranose
Chromose C	2,6-dideoxy-D-*arabino*-hexose
Chromose D	2,6-dideoxy-3-*O*-acetyl-D-*lyxo*-hexose

Table 4

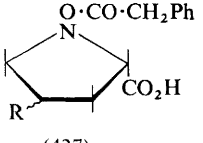
Chromomycin A_2	A-D-CHR-C-C-B
Chromomycin A_3	A-D-CHR-C-C-B′
Chromomycin A_4	CHR-D-C-C-A

Amaromycin has been shown to be identical with pikromycin, which contains desosamine as a component.[593]

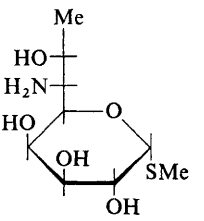
Full details of the previously briefly reported structural studies on lincomycin (435) have been described in a series of four papers.[354] The partial synthesis of a series of 4′-alkyl analogues (436) of lincomycin has been



(435) R = Pr
(436) R = other alkyl than Pr



(437)

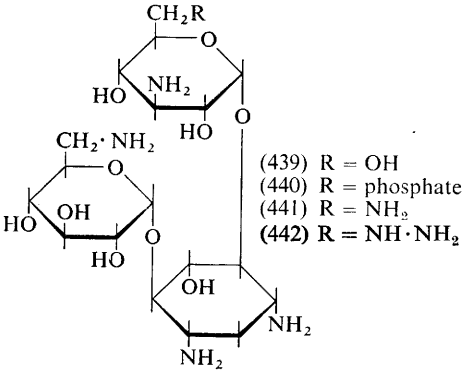


(438)

achieved by condensing compounds of type (437) with the thioglycoside (438).[594] The products were found to have enhanced antibacterial activity with respect to the natural antibiotic. The preparation of '4′-depropyl-4′-ethoxylincomycin' (*cis*- and *trans*-isomers) has also been reported.[595] Tritiation of lincomycin has been found to occur most satisfactorily by its exposure to tritium in the presence of platinum black. The distribution of the isotope was influenced by the exposure conditions.[596]

[592] M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara, K. Tanaka, S. Tatsuoaka, and K. Nakanishi, *Tetrahedron* 1967, **23**, 421.
[593] H. Ogura, A. Otagoshi, Y. Sano, and T. Hata, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 682.
[594] B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Medicin. Chem.*, 1967, **10**, 355.
[595] B. J. Magerlein, *J. Medicin. Chem.*, 1967, **10**, 1161.
[596] R. C. Thomas, G. J. Ikeda, and H. Harpootlian, *J. Pharm. Sci.*, 1967, **56**, 862.

Several papers have appeared on the chemical modification of kanamycin (439). Treatment of the tetra-*N*-acetyl derivative with benzaldehyde and zinc chloride, followed by deacetylation, gave the 4′,6′-*O*-benzylidene derivative.[597] Preferential phosphorylation with diphenylphosphochloridate of the tetra-*N*-anisylidene derivative followed by hydrolysis and hydrogenolysis gave kanamycin 6′-phosphate (440).[598,599] Both (440) and the 4′,6′-*O*-benzylidene derivative showed reduced antibacterial action when



(439) R = OH
(440) R = phosphate
(441) R = NH_2
(442) R = NH·NH_2

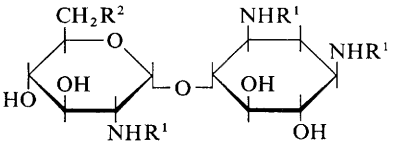
compared with the parent substance. The penta-amino-(441) and the hydrazino-(442) derivatives of kanamycin have been prepared by standard routes involving displacement of the 6′-toluene-*p*-sulphonyloxy-group.[600]

The deactivation of kanamycin and paromamine that results from treatment by an *E. coli* enzyme has been shown to be due to specific phosphorylation at the C-3-hydroxy-group in the 6-amino-6-deoxy-D-glucose and the 2-amino-2-deoxy-D-glucose moeities, respectively;[601] phosphatase treatment restored the activity. Another kanamycin-resistant *E. coli* that inactivated the antibiotic in the presence of ATP, co-enzyme A, and acetate acted by specific *N*-acetylation of the amino-group in the 6-amino-6-deoxy-D-glucose unit.[602] The deactivated compound was isolated chromatographically and identified.

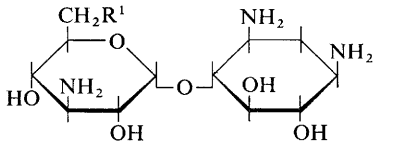
Neamine (443) has been prepared from tri-*N*-acetylparomamine (444) by preferential toluene-*p*-sulphonylation of the primary hydroxy-group,

[597] K. Tatsuta, K. Kobayashi, and S. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A.*, 1967, **20**, 267.
[598] S. Umezawa, K. Tatsuta, T. Tsuchiya, and E. Yamamoto, *Bull. Chem. Soc. Japan*, 1967, **40**, 1972.
[599] K. Tatsuta, T. Tsuchiya, E. Yamamoto, and S. Umezawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1967, **20**, 232.
[600] S. Inouye, *J. Antibiotics* (*Japan*), *Ser. A*, 1967, **20**, 6.
[601] H. Umezawa, M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda, and S. Mitsuhashi, *Science*, 1967, **157**, 1559.
[602] H. Umezawa, M. Okanishi, R. Utahara, K. Maeda, and S. Kondo, *J. Antibiotics* (*Japan*), *Ser. A*, 1967, **20**, 136.

displacement with azide ion, and then standard sequences. Since paromamine has previously been synthesised, the above conversion can be considered to represent the total synthesis of neamine.[603, 604a] 6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)deoxystreptamine (445), obtained by partial hydrolysis of kanamycin, has been converted by a similar sequence



(443) $R^1 = H$, $R^2 = NH_2$
(444) $R^1 = NHAc$, $R^2 = OH$



(445) R = OH
(446) $R = NH_2$

of reactions into the structural isomer of neamine (443), namely, 6-*O*-(3,6-diamino-3,6-dideoxy-α-D-glucopyranosyl)deoxystreptamine (446),[604a] which had no antibacterial properties.

The synthesis of daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) [363] and of its D-enantiomorph [364] have been reported. A number of synthetic analogues of the nucleoside antibiotic tubercidin (7-deaza-adenosine) have been described.[604b]

Methods for the quantitative analysis of the carbohydrate components of actinoidins A and B [605] and of the components of neomycin [606] have been reported.

Mass spectrometry has been applied to the intact aminocyclitol antibiotic, paramomycin, as its trimethylsilyl derivative. The sequence of the units could be established from the fragmentation pattern, showing that this technique may have important applications in structural work.[607] The optical rotatory dispersion spectra of *N*-salicylidene derivatives of several amino-sugar-containing antibiotics have been discussed.[392] Pyrolysis-gas chromatography has been used for distinguishing between, and characterising, various carbohydrate-containing antibiotics, which cannot themselves be chromatographed directly.[608]

[603] S. Umezawa, K. Tatsuta, T. Tsuchiya, and E. Kitazawa, *J. Antibiotics* (*Japan*), *Ser. A*, 1967, **20**, 53.
[604a] K. Tatsuta, E. Kitazawa, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1967, **40**, 2371.
[604b] J. F. Gerster, B. Carpenter, R. K. Robins, and L. B. Townsend, *J. Medicin. Chem.*, 1967, **10**, 326.
[605] F. Starichkai, N. N. Lomakina, I. A. Spiridonova, M. S. Yurina, and M. Pushkash, *Antibiotiki*, 1967, **12**, 126.
[606] M. K. Majumdar and S. K. Majumdar, *Analyt. Chem.*, 1967, **39**, 215.
[607] D. C. De Jongh, J. D. Hribar, S. Hanessian, and P. W. K. Woo, *J. Amer. Chem. Soc.*, 1967, **89**, 3364.
[608] T. F. Brodasky, *J. Gas Chromatog.*, 1967, **5**, 311.

21
Nucleosides*

A review on imidazole nucleosides has appeared,[609] and the work on nucleosides described during 1966 has been summarised.[610]

Naturally occurring Nucleosides

Chemical and spectroscopic evidence has shown that the broad-spectrum antibiotic showdomycin is a *C*-nucleoside, namely 3-(β-D-ribofuranosyl)-maleimide (433)[585, 586] which has been confirmed by an *X*-ray crystal structure study of its derivatives.[587]

Zeatin riboside [6-(*trans*-3-methyl-4-hydroxybut-2-enyl)amino-9-(β-D-ribofuranosyl)purine], previously found only in higher plants has been isolated from the puffball fungus and has for the first time been obtained crystalline.[611] The related nucleoside, 6-(3-methylbut-2-enyl)amino-9-(β-D-ribofuranosyl)purine, has been isolated[612] from yeast *s*-RNA (cf. reports on this compound during 1966).

Although not a nucleoside, the antibiotic aristeromycin, has been shown[588] to have the closely related structure (434).

Synthesis

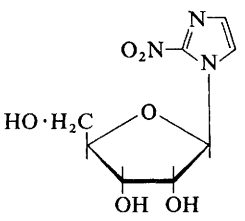
This continues to be a topic of great interest. In an attempt to simulate prebiotic synthesis of nucleosides, D-ribose and its 2-deoxy-derivative have been fused with bases.[613] The latter sugar and adenine gave a product whose u.v. spectrum and chromatographic properties were similar to those of 2′-deoxyadenosine, but its lability to alkaline hydrolysis showed that it was not this compound. It was tentatively suggested that it was a 9,3′-linked compound. Previous reports of the formation of 2′-deoxyadenosine were questioned.[613] Full details of the condensation of D-ribose and adenine in DMF in the presence of phenyl polyphosphate have now

[609] L. B. Townsend, *Chem. Rev.*, 1967, **67**, 533.
[610] K. S. Kirby and T. L. V. Ulbricht, *Ann. Reports*, 1966, **63**, 536.
[611] C. O. Miller, *Science*, 1967, **157**, 1055.
[612] M. J. Robins, R. H. Hall, and R. Thedford, *Biochemistry*, 1967, **6**, 1837.
[613] C. Reid, L. E. Orgel, and C. Ponnamperuma, *Nature*, 1967, **216**, 936.

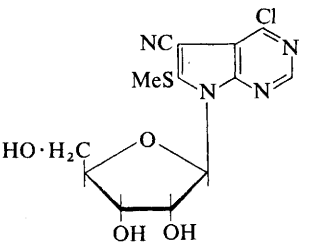
* For the terms of reference used for this Section, see the Introduction.

been reported.[614] Chromatographic separation of the products gave α- and β-adenosine in a combined yield of 18–20% (α : β approx. 1 : 1). 2′-Deoxy-D-ribose gave a combined α–β yield of nucleosides of 35–40% in which the α-anomer predominated.

The following compounds have been prepared by the fusion method; the ribofuranosyl derivative (447) of the antibiotic azomycin;[615] 2,6-dichloro-9-(2′,3′,4′-tri-*O*-acetyl-β-D-ribofuranosyl)purine;[616] several 2-substituted N^6-methyl-adenosines;[617] 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (448).[618] The fusion



(447)



(448)

method without catalyst has been investigated, and for 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 6-chloro-, 2,6-dichloro-, 6-cyano, or 2,6,8-trichloro-purine the yields were little different from those obtained when a catalyst had been used.[619] Use of a solvent such as anisole or nitromethane with the usual components employed in the fusion synthesis gave reasonable yields in the presence of an acid catalyst, but the autocatalytic reactions gave poor yields, much worse than from the fusion procedure.[620]

The syntheses of nucleoside derivatives of amino-sugars have appeared using 2,4-dinitrophenyl,[87, 621, 622] trichloroacetyl,[385] trifluoroacetyl,[385] and bisphenoxyphosphinyl[384] as amino-blocking groups on the esterified amino-glycosyl halide.

The use of glycals as the carbohydrate moiety in 2′-deoxy-nucleoside synthesis has been extended to the synthesis of 9-(2′-deoxy-3′,4′-di-*O*-acetyl-β-D-*erythro*-pentosyl)adenine starting from 3,4-di-*O*-acetyl-D-arabinal.[475]

[614] G. Schramm, G. Lunzmann, and F. Bechmann, *Biochim. Biophys. Acta*, 1967, **145**, 221.
[615] R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 1967, **4**, 311.
[616] Y. H. Pan, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 1967, **4**, 246.
[617] G. Gough and M. H. Maguire, *J. Medicin. Chem.*, 1967, **10**, 475.
[618] R. L. Tolman, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 1967, **4**, 230.
[619] Y. Ishido, T. Matsuba, A. Hosono, K. Fujii, T. Sato, S. Isome, A. Maruyama, and Y. Kikuchi, *Bull. Chem. Soc. Japan*, 1967, **40**, 1007.
[620] Y. Ishido, H. Tanaka, T. Yoshino, M. Sekiya, K. Iwabuchi, and T. Sato, *Tetrahedron Letters*, 1967, 5245.
[621] M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, 1967, **32**, 1823.
[622] M. L. Wolfrom and H. B. Bhat, *J. Org. Chem.*, 1967, **32**, 2757.

A number of papers have appeared on the $O \rightarrow N$-migration that occurs during nucleoside formation from silver salts of bases.[87-89, 623-626]

The syntheses of the following nucleoside derivatives have also been described during 1967: 2-deoxy-D-*arabino*-hexopyranosyl derivatives of 6-aza-uracil and -thymine,[627] 9-(β-D-arabinofuranosyl)adenine,[628] 1-(β-D-arabinofuranosyl)-5-methylcytosine [629] and its 5-fluoro-analogue,[630] 1-(β-D-arabinofuranosyl)-5-fluorouracil,[630] 3′-deoxy-β-D-*xylo*-hexofuranosylthymine and its pyranose analogue,[631] several 3′-*O*-methyl-ribofuranosyl nucleosides,[632] 9-(β-L-sorbopyranosyl)adenine [633] and its 1′-deoxy-analogue,[634] [9-(β-D-mannofuranosyl)adenine],[337] the eight possible 9-(tetrofuranosyl)adenines,[635] 9-(β-melibiosyl)adenine,[636] 9-(α-D-ribofuranosyl)-adenine,[637] several 3′-deoxy-D-*erythro*-pentofuranosylguanine nucleosides,[638] the anomeric 3-(2′-deoxy-D-*erythro*-pentofuranosyl)adenines,[639a] and 9-(2′deoxy-β-D-*erythro*-pentofuranosyl)adenine and its 3′-deoxy-analogue.[639b]

The synthesis of a *xylo*-nucleoside analogue with an acetimido-group replacing the ring oxygen has been reported.[248] A branched-chain nucleoside (340), an isomer of adenosine, has been synthesised.[498] The synthesis of the *lyxo*-nucleoside (150), the last of the series of 9-(β-D-pentofuranosyl)-adenines to be prepared, has been described.[320] The 'reverse nucleoside' derivative (215) has been prepared from methyl 5-amino-5-deoxy-β-D-ribofuranoside.[310]

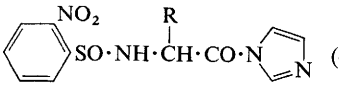
Isolation of a number of *C*-nucleosides in natural products in recent years has stimulated an interest in their laboratory synthesis. 3-(β-D-Ribofuranosyl)-2,6-dibenzyloxypyridine (35) has been prepared by condensation of the 3-cadmium derivative of the base with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride, followed by debenzoylation.[147] Removal of the benzyl groups gave 1-deazauridine which was chemically unstable. Another route has used hexulosonic acid thiosemicarbozones as intermediates.[148]

[623] I. A. Mikhailopulo, V. I. Gunar, and S. I. Zavialov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 1811.
[624] I. A. Mikhailopulo, V. I. Gunar, and S. I. Zavialov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 470.
[625] H. Pischel and G. Wagner, *Z. Chem.*, 1967, **7**, 15.
[626] G. Schmidt and J. Farkas, *Tetrahedron Letters*, 1967, 4251.
[627] G. J. Durr, J. F. Keiser, and P. A. Ierardi, *J. Heterocyclic Chem.*, 1967, **4**, 291.
[628] F. Keller, I. J. Botvinick, and J. E. Bunker, *J. Org. Chem.*, 1967, **32**, 1644.
[629] I. L. Doerr, J. F. Codington, and J. J. Fox, *J. Medicin. Chem.*, 1967, **10**, 247.
[630] F. Keller, J. E. Bunker, and A. R. Tyrrill, *J. Medicin. Chem.*, 1967, **10**, 979.
[631] E. Wittenburg, *Z. Chem.*, 1967, **7**, 13.
[632] G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, 1967, **32**, 1984.
[633] H. Paulsen, H. Köster, and K. Heyns, *Chem. Ber.*, 1967, **100**, 2669.
[634] J. Farkas and F. Sorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2663.
[635] D. H. Murray and J. Prokop, *J. Pharm. Sci.*, 1967, **56**, 865.
[636] L. M. Lerner, *J. Org. Chem.*, 1967, **32**, 3663.
[637] K. Onodera, S. Hirano, and F. Masuda, *Carbohydrate Res.*, 1967, **4**, 263.
[638] G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, *J. Org. Chem.*, 1967, **32**, 859.
[639a] M. Rasmussen and N. J. Leonard, *J. Amer. Chem. Soc.*, 1967, **89**, 5439.
[639b] M. Ikehara and H. Tada, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 94.

Esters

A large number of papers have appeared on the synthesis of nucleoside phosphates, both enzymic,[291] and chemical,[279-287, 289] including one on a method in which a ribose phosphate derivative is formed before condensation with the base,[640] and one [288] on the synthesis of bis(pyrimidine nucleoside) phosphates. A detailed kinetic study has been made of the nonenzymic hydrolysis of cyclic 2′,3′-phosphates.[290] The synthesis of nucleoside phosphites has been reported.[292]

The use of isobutylcarbonate,[257] the *p*-nitrophenylcarbonate,[256] and the adamantoate [240] esters as blocking groups for synthesis of nucleoside derivatives, particularly phosphates, has been described. Nitrate,[334a] sulphate,[301] and trifluoroacetate [236] esters of nucleosides have also been reported, as has a study on acetyl migration in derivatives of 3-(β-D-xylofuranosyl)uracil.[233] Esterification of nucleosides and nucleotides with the reagent (449) has provided a new method of preparing 2′- and 3′-aminoacyl derivatives.[641]



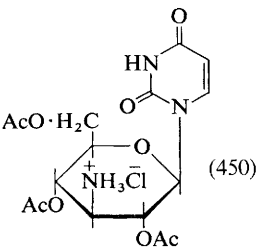
(449)

The n.m.r. spectra of a wide variety of acetylated nucleosides have been discussed (see p. 183).

Other Nucleoside Derivatives

The nitromethane cyclisation reaction has been used with the dialdehyde from the periodate oxidation of cytidine,[432] of 3-(β-D-glucopyranosyl)-thymine [430] and of 7-(β-D-ribofuranosyl)- and 7-(β-D-glucopyranosyl)-theophylline [431] to give 3′-deoxy-3′nitropyranosyl nucleosides, and thence the 3′-amino-3′-deoxy-derivatives.

N-Aminoacyl compounds have been prepared by coupling of the uracil derivative (450) with *N*-carbobenzoxy-derivatives of amino-acids.[432]



(450)

[640] M. Asai, M. Miyaki, and B. Shimizu, *Agric. and Biol. Chem.* (*Japan*), 1967, **31**, 319

[641] B. P. Gottikh, A. A. Kraevsky, P. P. Purygin, T. L. Tsilevich, Z. S. Belova, and L. N. Rudzite, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1967, 2571.

The 4′,5′-unsaturated nucleosides (333)[488] and (331)[487] have been prepared, the latter as an analogue of angustmycin (332). 2′,3′-Dideoxy- and 2′,3′-dideoxy-2,3′-didehydro-derivatives of cytidine have been synthesised,[470] as has the latter derivative of 5-fluorouridine.[483]

The use of complex neighbouring groups in the displacement of sulphonyloxy-groups has led to the synthesis of 3′-thioadenosine[315] and of 3′-amino-3′-deoxy-2′-thiouridine.[314] Nucleophilic attack on the two epimeric 5′-deoxyadenosine 2′,3′-epoxides (47) and (48) with sodium benzylsulphide and with sodium azide gave, after standard conversions, deoxy- and amino-deoxy-derivatives respectively.[187]

Tri-*O*-trityluridine has been described.[168] The methoxymethylidene group has been used as a blocking group for the *cis*-2′,3′-diol group in nucleosides.[255] The mixed acetal obtained by reaction of 4-methoxy-5,6-dihydro-2*H*-pyran with a nucleoside hydroxy-group has been proposed as the best available blocking group for oligonucleotide synthesis[226] and, unlike the similar tetrahydropyranyl blocking group, it has the advantage of being symmetrical. 2′,3′-*O*-Benzylideneuridine derivatives have been described in which the aromatic ring carries nitrogen-mustard groupings.[642]

Oxidation of the appropriate di-*O*-trityl derivative with the Pfitzner–Moffatt reagent or with DMSO and acetic anhydride gave the 2′- and 3′-keto-nucleosides (348) and (347). Borohydride reduction gave predominantly the *xylo*- and *arabino*-products respectively.[508] Fox and his colleagues have continued their study of 2′-deoxy-2′-halogeno-nucleosides with the synthesis of 2′-chloro- and 2′-fluoro-2′-deoxy-derivatives of cytidine, from the corresponding uridine derivatives.[643]

The synthesis of 5′-deoxyuridine 5′-phosphonic acid has been described.[548] Two papers[545, 546] have appeared on cobalt derivatives of adenosine, and one on the complexes of nucleosides with boric acid.[551]

[642] A. M. Belikova, V. F. Zarytova, and N. I. Grineva, *Tetrahedron Letters*, 1967, 3557.
[643] I. L. Doerr and J. J. Fox, *J. Org. Chem.*, 1967, **32**, 1462.

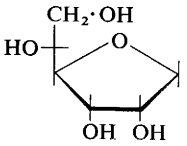
22
Oxidation* and Reduction

Periodate Oxidation

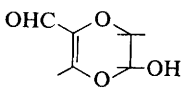
A review has appeared on the organic chemistry of preriodates in which mechanism, stereochemistry, and products of cleavage reactions are discussed.[644]

A detailed study of the oxidation of malonaldehyde with periodate has been undertaken[645] with a view to devising methods for the analysis of deoxy-sugars. It was shown that malonaldehyde did not react stoicheiometrically with periodate in the pH range 1 to 8. However, at 4° in 0·1N-sulphuric acid it was not oxidised, and so it was recommended that the oxidation of diol groups in deoxy-sugars should be carried out under those conditions. Malonaldehyde can be estimated by colorimetric analysis after reaction with thiobarbituric acid.

An important contribution to the understanding of the overoxidation that occurs on prolonged periodate oxidation of many carbohydrate derivatives has been made by the characterisation of compound (452) from the oxidation of 1,4-anhydro-D-allitol (451).[646] Overoxidation of



(451)



(452)

(451) occurred *via* hydroxylation of the double bond in (452) and subsequent glycol cleavage. Compound (452) was obtained crystalline and characterised chemically and spectroscopically. The reactions were discussed in detail, and the overoxidation of several carbohydrate compounds was interpreted in terms of intermediates analogous to (452).

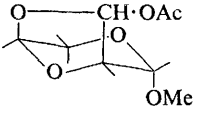
A detailed study on the periodate oxidation of the α-dicarbonyl series, glyoxal, pyruvaldehyde, and butane-2,3-dione has been reported;[647] the

[644] B. Sklarz, *Quart. Rev.*, 1967, **21**, 3.
[645] P. Szabo and L. Szabo, *Carbohydrate Res.*, 1967, **4**, 206.
[646] B. G. Hudson and R. Barker, *J. Org. Chem.*, 1967, **32**, 2101.
[647] G. Dahlgren and K. L. Reed, *J. Amer. Chem. Soc.*, 1967, **89**, 1380.

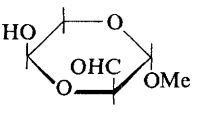
* See also Section 16.

findings provide the basis for understanding oxidations that have been reported in the carbohydrate field.

Oxidation of methyl glycopyranosides in DMSO instead of the usual aqueous medium, used only one molar equivalent of periodate, instead of the expected two.[648] The product obtained after treatment of methyl β-L-arabinopyranoside with periodate in DMSO, followed by acetylation, gave an n.m.r. spectrum consistent with structure (453). It was believed that in all cases, the products after cleavage of one diol group formed



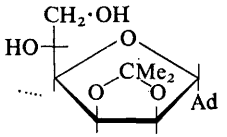
(453)



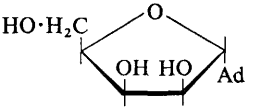
(454)

internal hemiacetals [such as (454) for methyl β-L-arabinoside] that could not be oxidised further. *cis*-Diol groups were, in general, preferentially oxidised, but there was found to be a tendency for the C-2—C-3 bond to be cleaved in pentosides (*e.g.* methyl xylosides and ribosides), and for the C-3—C-4 bond to be surprisingly reactive in hexosides (methyl α-D-mannopyranoside, for example, was oxidised nonselectively). Comparison of 1-H—2-H spacings in the n.m.r. spectra of the glycosides in DMSO and in deuterium oxide suggested that a solvent-induced conformational change was not an important factor in determining the differences in oxidation in the two solvents.

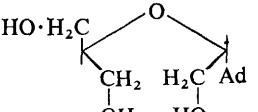
Periodate oxidation has been used in the proof[649] of the structure of a mannofuranosyl nucleoside (454A), shortening the side-chain by one carbon atom to give, after removal of the acetal group and reduction, the lyxofuranosyl derivative (455). This product was then oxidised again with



(454A)



(455)



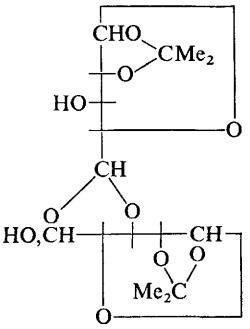
(456)

periodate followed by reduction with borohydride to give the triol (456)' which was enantiomeric with the triol similarly obtained from adenosine, establishing that the anomeric configuration in (454A) was α.

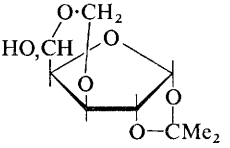
The periodate oxidation of 1,2-*O*-isopropylidene-D-glucofuranose gave bis-(5-aldo-1,2-*O*-isopropylidene-α-D-*xylo*-pentofuranose)-5,5′:3,5′-cyclic

[648] R. J. Yu and C. T. Bishop, *Canad. J. Chem.*, 1967, **45**, 2195.
[649] P. Kohn, L. M. Lerner, and B. D. Kohn, *J. Org. Chem.*, 1967, **32**, 4076.

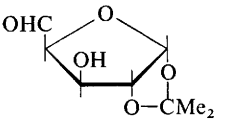
acetal (457) as the major product, together with 5-hydroxy-1,2-*O*-isopropylidene-3,5-*O*-methylene-α-D-xylofuranose (458).[650] Oxidation with lead tetra-acetate gave (458) as the major product, with (457) as the minor product. Neither oxidant gave the free aldehyde (459). The methylene acetal group in (458) was thought to arise from condensation of (459) with the formaldehyde produced during the oxidation.



(457)



(458)



(459)

The products from periodate oxidation of *N*-acetyl-4,6-*O*-benzylidene-*N*-(*p*-methoxyphenyl)-β-D-glucopyranosylamine and the *p*-phenylazo-analogue are believed to exist as mixtures of the free dialdehyde and cyclic solvated forms.[651]

Attention has been drawn [652] to a possible error in the determination of formate from the periodate oxidation of labelled carbohydrates, the products from which have been reduced with borohydride and excess of reagent decomposed with carbon dioxide. It was shown that some carbon dioxide was reduced to formate by the borohydride. Hence, with labelled borohydride, more than the expected amount of formate was determined. A microanalytical method for the determination of periodate has been described (see p. 196).

Platinum-catalysed Oxidation

Work on the platinum-catalysed selective oxidation of carbohydrate derivatives has been continued by the Hamburg school. All eight possible 1,6-anhydro-β-D-hexopyranosides are now available, and so a comparison of the relative ease of oxidation of various hydroxy-groups has been made.[191] The order of reactivity was found to be 3-*ax* > 4-*ax* > 2-*ax* > 4-*eq* > 2-*eq* > 3-*eq*. The oxidation products were characterised by reduction.

[650] T. D. Inch, *Carbohydrate Res.*, 1967, **5**, 53.
[651] Z. Fialkiewiczowa and M. Kalmanowa, *Roczniki Chem.*, 1967, **41**, 51.
[652] F. Eisenberg, jun., and A. H. Bolden, *Carbohydrate Res.*, 1967, **5**, 349.

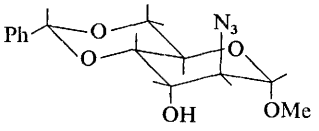
Platinum-catalysed oxidation of 1,2-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose gave a 3-keto-derivative, leaving the C-5-hydroxy-group unoxidised.[510] In the 3-deoxy-analogue, the C-5-hydroxy-group was again not oxidised. Removal of the toluene-*p*-sulphonyl group, however, gave products that were oxidised. It was suggested, therefore, that an adjacent toluene-*p*-sulphonyl ester group inhibited catalytic oxidation. (It should be noted that no structural proof was given for any of the products in this paper.) Selective oxidation of an axial hydroxy-group has been used in a synthesis of streptamine.[576]

DMSO-based Oxidations

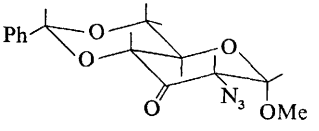
The use of such oxidations has been widely reported,[155, 190, 303, 474, 481, 505–510, 530, 531, 632] and a review on the topic has appeared.[504] The DMSO–acetic anhydride system has been used as the key step in a new synthetic approach to ketoses.[197] The readily available benzylated aldopyranoses or furanoses with benzyl ether groups at all positions except C-1 were reduced to the alditol ethers, C-1 was then blocked selectively, and the freed secondary hydroxy-group was oxidised. Removal of the blocking group then gave the ketose. For example, L-sorbose was prepared from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose in 33% yield (a trityl ether group was used to protect C-1 in the glucitol). This method should be useful for the synthesis of terminally substituted ketoses.

Oxidation of several inositols with DMSO–acetic anhydride in the presence of pyridine led to a good yield of penta-acetoxybenzene. Experiments with inositol derivatives suggested that the reaction proceeded *via* the intermediate formation of a diketoinositol.[565]

Epimerisation accompanying oxidation, first observed in 1965, has now been shown to occur when an axial azido-group was adjacent to the site of oxidation. Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altroside (460)



(460)



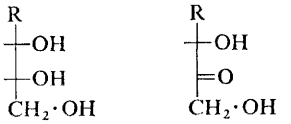
(461)

with DMSO and acetic anhydride gave the C-2-equatorial azido-product (461).[305, 424] Experiments with labelled systems suggested[424] that the epimerisation occurred after oxidation by an acid-catalysed enolisation mechanism.

Other Oxidations

It is well established that *Acetobacter suboxydans* generally oxidises the grouping (462) to (463). Papers have appeared, however, which show that departures from this behaviour can occur. D-Galactose diethyl dithioacetal

and the dimethyl acetal were oxidised at position 5 and not, as expected, at position 3;[653] 1-deoxy-D-galactitol was, however, oxidised at positions 3 and 5. D-Galactitol and several other closely related compounds were not oxidised.



(462) (463)

Another report[654] stated that different strains of the organism showed different oxidising behaviour, and that each strain contained more than one dehydrogenase. Strains were examined which oxidised *cis*- and *trans*-cyclohexane-1,2-diols and *cis-cis*-cyclohexane-1,2,3-triol and, whereas particulate fractions showed a pH optimum at 5·1–5·3 and had *R* specificity, soluble fractions had a pH optimum in the range 8–10 and had *S* specificity (see Scheme 43). The combined properties were shown by intact bacteria, so that enantiomeric 2-hydroxy-cyclohexanones were formed from cyclohexane-1,2-diols at acid and alkaline pH values. One organism oxidised *scyllo*-inositol (as shown in Scheme 44) independent of pH.

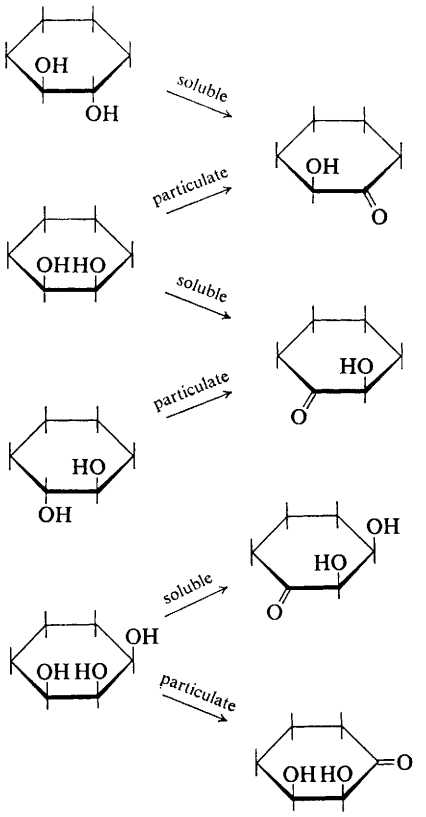
The oxidation of a series of cyclohexane-1,2,3,4-tetrols by *Acetobacter suboxydans* has been shown to occur at axial hydroxy-groups.[567] In some cases the initial products were converted to epimers by the action of epimerases. The enantiomeric mono-*O*-methyl-cyclohexane-1,2,3-triols were oxidised as shown in Scheme 45 (see p. 178).

Acetobacter suboxydans converted D-sorbitol into L-sorbose and then further into 5-keto-D-fructose, the maximum yield of which was above 70% after 20 hr. This product was further converted into three γ-pyrone derivatives, namely kojic acid, 3-oxykojic acid, and 5-oxymaltol.[655]

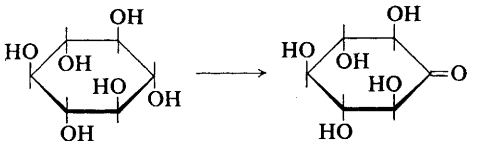
A kinetic study has been made of the oxidation of melibiose, cellobiose, lactose, and maltose by alkaline ferricyanide;[656a] the reaction, which was found to be first order with respect to oxidant, was assumed to proceed *via* oxidation of a 1,2-enediol, the formation of which was rate determining.

A wide variety of organic compounds have been shown to be oxidised by silver(II) picolinate in solvents such as water–DMSO or DMSO–ethylene glycol dimethyl ether.[520] The oxidation of the following carbohydrate derivatives was reported: 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, 2,3:4,5-di-*O*-isopropylidene-D-fructopyranose, and 2,3:4,6-di-*O*-isopropylidene-L-sorbofuranose

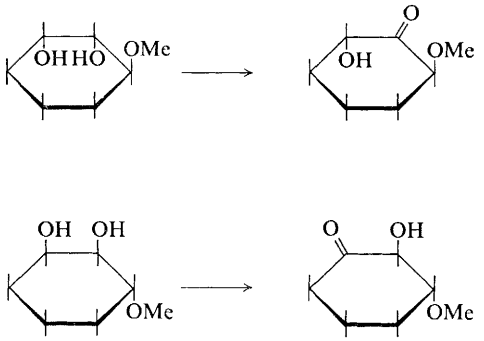
[653] D. T. Williams and J. K. N. Jones, *Canad. J. Chem.*, 1967, **45**, 741.
[654] A. Rapin, A. L. Haenni, and T. Posternak, *Helv. Chem. Acta*, 1967, **50**, 1801.
[655] K. Sato, Y. Yamada, K. Aida, and T. Uemura, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 877.
[656a] R. K. Srivastava, N. Nath, and M. P. Singh, *Tetrahedron*, 1967, **23**, 1189.



Scheme 43



Scheme 44

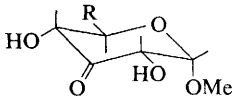


Scheme 45

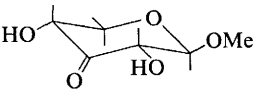
all gave the corresponding acids; 3-*O*-benzoyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol and methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside gave the corresponding keto-compounds.

Reduction

The borohydride reduction of the keto-group in aldulose and ketulose derivatives (generally prepared by DMSO oxidations) has been used for the synthesis of the epimers of the starting alcohols. In most cases the reduction was stereospecific. Reduction of the di-*O*-isopropylidene diulose (prepared from 1,2:4,5-di-*O*-isopropylidene-D-fructose)[505, 506] or its di-*O*-cyclohexylidene analogue[506] gave, after removal of the blocking groups, D-psicose (see Scheme 36). Reduction of the methyl D-*erythro*-pentopyranosid-3-uloses has been studied:[507] the α-anomer (464) gave the



(464) R = H
(465) R = $CH_2 \cdot OH$



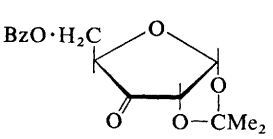
(466)

riboside and xyloside in the ratio 24 : 1, whilst the β-anomer (466) gave a ratio of 0·82 : 1. Reduction[507] of methyl α-D-*ribo*-hexopyranoside-3-ulose (465) gave the *allo*- and *gluco*-products in the ratio of 66 : 1. These results demonstrated the controlling steric effect of the axial methoxy-group in the α-anomers.

The nucleoside derivatives (347) and (348) on borohydride reduction both gave products that resulted from predominant attack from the α-face of the molecule, that is with *xylo*- and *arabino*-configurations, respectively.[508] Reduction of compound (467) gave the product with the *ribo*-configuration with high specificity;[632] the acetal group presumably directs the reduction in the opposite way to that of (347). This result was similar to the reduction

of the derivative (468) to the *allo*-product (ref. 506 and several previous reports of this reduction in 1966).

The ethanol-modified lithium aluminium hydride complex of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose has been used in the asymmetric reduction of ketones, giving products of 70% optical purity with the *R*-configuration.[656b]



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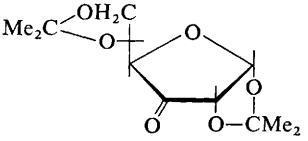


(468)

Azides have been shown to be readily reduced to amines by sodium borohydride in methanolic DMF.[424]

The reduction of lactones has been mentioned (p. 143).

[656b] S. R. Landor, B. J. Miller, and A. R. Tatchell, *J. Chem. Soc.* (*C*), 1967, 197.

23

N.m.r. Spectroscopy and Conformational Features of Carbohydrates

Two general articles of considerable interest to carbohydrate chemists have appeared in *Quarterly Reviews*. Robinson and Theobald [657] have discussed cyclohexane derivatives which adopt abnormal ground-state conformations, and make it clear that many non-chair pyranoid rings will be encountered in carbohydrate chemistry. Riddell [658a] has reviewed the conformational analysis of heterocyclic compounds: steric and dipolar factors were considered, as were intramolecular hydrogen-bonding and lone-pair 'volume'.

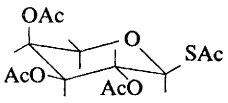
No major advance has been made in carbohydrate stereochemistry which can be recorded specifically under the latter part of the above heading, but a large number of interesting points have nevertheless been noted. These have, in the main, been observed by n.m.r. spectroscopic techniques so they are summarised in this combined section. Of pre-eminent interest are (i) the observation that the n.m.r. spectrum of tetra-*O*-acetyl-β-D-ribopyranose was resolved at low temperature into those of the two chair conformations and so, at room temperature, the compound was shown to be in continuous motion between them,[228] and (ii) the finding that the anomeric effect was sufficiently strong in acetylated xylopyranosyl halides to hold the β-anomer in the all-axial chair form.[339, 341] The anomeric effect of glycosyl halides is discussed in detail on p. 83, and its influence in determining conformations of unsaturated sugar derivatives has also been mentioned.[483]

Study of free sugars in solution has allowed pyronose⇌furanose equilibria to be investigated;[19] a method based on analysis of long-range coupling can be applied to determination of the anomeric configuration in ketoses.[25] The broad-line spectrum of crystalline α-D-glucopyranose has been investigated.[26]

A striking development has been the application, for the first time, of 220 Mc./sec. instruments and the demonstration that the increased resolving power which these afford can allow solutions to problems which were

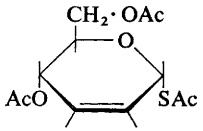
[657] D. L. Robinson and D. W. Theobald, *Quart. Rev.*, 1967, **21**, 314.
[658a] F. G. Riddell, *Quart. Rev.*, 1967, **21**, 365.

insoluble even by the use of 100 Mc./sec. spectrometers.[341, 342, 557, 658b, 659] Thus the 100 Mc/sec. spectrum of a 1-thio-L-arabinose tetra-acetate was not interpretable, but with the 220 Mc./sec. instrument a clearly resolved spectrum was obtained, which revealed that the compound had the α-configuration and the *C*1 conformation (469).[659]



(469)

In a further report Horton and his group[658b] have described spectra obtained in deuteriochloroform at 60, 100, and 220 Mc./sec. of several 1-thio-aldose peracetates. The derivatives of β-D-xylose, α-L-arabinose, β-D-ribose, β-D-glucose, and β-D-galactose were all examined. First-order spectra were obtained for each compound and all were found to adopt the *C*1 chair conformation. The deshielding effect of the thioacetyl group was seen to be less than that of the acetoxy-group since 1-H signals for the sulphur compounds occurred 0·35 p.p.m. upfield from those in the spectra of the oxygen analogues. Further, the anomeric effect in the thio-derivatives was apparently less than that operating in the oxygenated compounds, since the β-*ribo*-thio-ester adopted the *C*1 chair form, whereas tetra-*O*-acetyl-β-D-ribopyranose existed predominantly in the 1*C* conformation.[228] In the same paper,[658b] it was shown that the unsaturated compound (470)



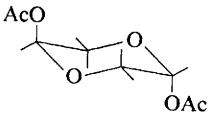
(470)

adopted the *H*1 half-chair conformation as expected, and the value of varying the solvent used for spectral measurements was well illustrated. For example, for tri-*O*-acetyl-β-D-ribopyranosyl chloride the 2-H and 3-H signals were not resolved in chloroform but were separated in benzene solution.[339] Long range and virtual coupling were encountered in this work and were discussed; solvent changes can remove the latter cause of multiplicities of signals. It was also observed that the 5-H (*ax*) signal occurred (unusually) downfield relative to that from 5-H (*eq*), as a consequence of the deshielding influence of the axial chlorine and the C-3-acetoxy-group.

[658b] C. V. Holland, D. Horton, M. J. Miller, and N. S. Bhacca, *J. Org. Chem.*, 1967, **32**, 3077.

[659] N. S. Bhacca and D. Horton, *Chem. Comm.*, 1967, 867.

Too many other compounds have been characterised conformationally by n.m.r. methods to be enumerated; several are referred to under other headings. One exception, however, is the acetylated *trans*-dimer of glycolaldehyde which has been shown to adopt the diaxial chair form (471) rather than the alternative chair adopted by the corresponding, and previously examined, trimethylsilylated ether, and so it can be concluded



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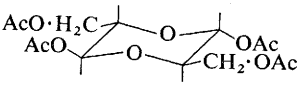


(472)

that (as might be expected) the anomeric effect of the trimethylsilyloxy-group is outweighed by steric factors, whereas the opposite holds for the acetate.[660] In the case of the acetylated dimer of DL-glyceraldehyde the isolated compound was the dioxan (472), *i.e.* it was strictly analogous to the trimethylsilyl ether.[660]

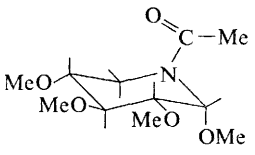
Since acetoxy- and acetamido-groups on pyranoid rings resonate at different fields depending upon their axial or equatorial orientation, chemical-shift measurements can reveal stereochemical features of acetylated carbohydrates. Horton and his co-workers have determined the chemical shifts of the *N*-acetyl protons in derivatives of 2-acetamido-2-deoxy-D-glucose and -mannose (τ 8·09 and 7·92–7·96, respectively), and also the shifts of the acetoxy-protons in a wide variety of 2-amino-2-deoxy-D-glucose compounds with the aid of specifically deuteriated esters. Aryl substituents were found to cause significant shielding of acetyl protons, so that a resonance near τ 8·09 in aromatic derivatives need not necessarily signify an equatorial acetamido-group.[235] Syntheses of specifically deuteriated acetates of 2-acetamido-2-deoxy-D-glucose were described, and the positions of resonance of individual ester groups characterised, so that n.m.r. investigation of partially acetylated compounds after deuterioacetylation will provide means for determining the position and degree of primary substitution.[661]

Lichtenthaler and Emig [662a] have studied the methyl resonances of acetoxy- and acetamido-groups at tertiary carbons (branch points), and concluded that the position of resonance again depended upon the axial or equatorial character of the group, so that knowledge of chemical shifts can provide means for determining configuration in branched-chain derivatives. This is particularly valuable since normal methods based on spin–spin coupling interactions are not applicable to these compounds. Resonances of the acetoxy-groups were found to be about 0·1 p.p.m. upfield relative to those at corresponding secondary sites.[662a]

[660] L. D. Hall, *Carbohydrate Res.*, 1967, **4**, 429.
[661] D. Horton, W. E. Mast, and K. D. Philips, *J. Org. Chem.*, 1967, **32**, 1471.
[662a] F. W. Lichtenhaler and P. Emig, *Tetrahedron Letters*, 1967, 577.

Despite the above reports, it has been claimed from the study of thirty-six acetylated amino-hexosyl and -pentosyl nucleosides that the use of the signals from acetoxy- and acetamido-groups cannot be used unambiguously for configurational assignments for carbohydrates in general and for nucleosides in particular.[662b]

N-Acetyl groups of *N*-acetylated derivatives of six-membered cyclic carbohydrates having nitrogen in the ring have received particular attention.[663] It is now well known that such groups show two n.m.r. signals corresponding to conformational isomers which do not interconvert at room temperature because of restricted rotation about the nitrogen to carbonyl bond. The anisotropic effect of this carbonyl group has now been shown to influence the chemical shift of protons on the *cis*-α-carbons. Equatorial protons were deshielded while axial atoms, conversely, were shielded. In the spectrum of the isomer (473), for example, the anomeric proton was almost 1 p.p.m. upfield relative to the signal from this proton in isomer (474), and similar effects were observed for the equatorial protons



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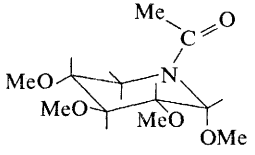

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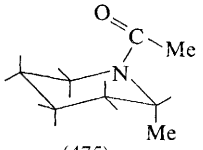

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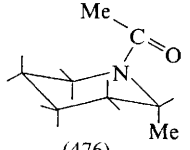


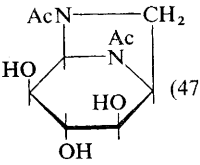
(476)

at C-5. This rationalisation has allowed ring conformational assignments to the conformational isomers (475) and (476) of *N*-acetyl-2-methylpiperidine. Both adopt the chair forms having the methyl group axial where it interacts less with the acetyl group than it would were it equatorial. Hindered rotation in the *N*-acetyl groups of compound (477) has been studied by ^{1}H n.m.r. methods at various temperatures. The coalescence temperatures were shown to be different for the two groups.[664]

Hall and his colleagues have made several important contributions to the understanding of spin–spin couplings in carbohydrate molecules. Couplings over four bonds on pyranoid rings have previously been recorded

[662b] R. J. Cushley, K. A. Watanabe, and J. J. Fox, *J. Amer. Chem. Soc.*, 1967, **89**, 394.
[662c] R. A. Long, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, 1967, **32**, 2751.
[663] H. Paulsen and K. Todt, *Chem. Ber.*, 1967, **100**, 3385.
[664] H. Paulsen and K. Todt, *Chem. Ber.*, 1967, **100**, 3397.

by several authors, and it has been recognised that the effects are strongest between pairs of equatorial protons. Now it has been shown that coupling constants for such systems are in the range 1·2–1·6 c./sec. (and are positive), whereas equatorial and axial protons have a coupling of 0·5 ± 0·2c./sec. (negative), so a method for distinguishing such pairs of protons is at hand and should be applicable with profit to configurational problems.



(477)

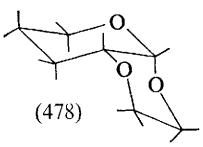
Long-range coupling was also observed across the ring oxygen of pyranoid compounds (1-H to 5-H) and in furanoid ring systems.[665] Preliminary results have been reported on investigations into the signs of proton–proton spin coupling constants for a number of types of proton pairs in saturated and unsaturated carbohydrates.[666] A detailed n.m.r. study has been made of the interesting acetal (57) and its diacetate. Considerable long-range coupling across four and five bonds was observed which made assignments difficult, particularly for the anomeric proton.[200]

Signs of vicinal and geminal ^{19}F, proton-coupling constants for nine glycosyl fluoride derivatives have been examined and all were found to be positive despite the report by other workers that such vicinal coupling constants can be negative.[667] A full report [342] has appeared on ^{19}F-proton interactions in glycosyl fluorides. 3,4,6-Tri-*O*-acetyl- and -benzoyl-2-deoxy-α-D-*arabino*-hexopyranosyl fluoride were prepared for this study by treatment of the corresponding tetra-esters with hydrogen fluoride. Couplings of 38 c./sec. were found for one 2-H–F interaction and consequently the fluorine can be defined as axial. Geminal coupling constants observed for several 2-deoxy-compounds conform with the generalisation that larger values are observed when a neighbouring electronegative substituent is axial than where it is equatorial. Full n.m.r. parameters were recorded (some measured at 220 Mc./sec.) for the two newly synthesised glycosyl fluorides, the corresponding tetra-acetates (α and β), the tetra-benzoate (β), and the anomeric 2,3,4,6-tetra-*O*-acetyl-α- and β-D-gluco-pyranosyl fluorides.

On the basis of considerations of nonbonded interactions the conformations of the hydroxymethyl groups of α-D-glucopyranose and α-D-galactopyranose have been computed mechanically. Similarly, the activation energy for the transition from the *C*1 chair to a boat has been determined.[668]

[665] L. D. Hall, J. F. Manville, and A. Tracey, *Carbohydrate Res.*, 1967, **4**, 514.
[666] L. D. Hall and J. F. Manville, *Carbohydrate Res.*, 1967, **4**, 271.
[667] L. D. Hall and J. F. Manville, *Chem. and Ind.*, 1967, 468.
[668] K. M. Grushetskii and A. F. Bestsennyi, *Zhur. strukt. Khim.*, 1967, **8**, 325, 332.

Other general points have been reported which are of significance in carbohydrate stereochemistry. Variable temperature study of the n.m.r. spectrum of tetrahydropyran has shown that the conformational mobility of the molecule is similar to that of cyclohexane, so the hetero-atom has little effect.[669] In work with other 'model carbohydrates' (478) and (479)



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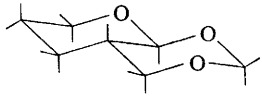


(479)

it has been shown that the ring-junction coupling constants were 1·3 c./sec. for the *cis*-compound and 7·1 c./sec. for the *trans*-isomer, and it was considered that these figures represent 'true' $J_{1,2}$ values for pyranose sugars and their derivatives.[670]

The steric requirements of the methyl and trideuteriomethyl groups have been compared by studying the *cis*, *trans* equilibrations of 4-t-butyl-2-methylcyclohexanone and its deuteriomethyl analogue by g.l.c. analysis. In each case, the *cis*-isomer was favoured to the same extent (possible error 0·05 kcal./mole) so no difference between the steric demands of the groups was detectable.[671]

Allylic groups on unsaturated pyranoid rings can favour the axial orientation.[483] In investigations of this phenomenon *cis*- and *trans*- 5-t-butylcyclohex-2-enol and the corresponding pairs of methyl ethers and acetyl esters were equilibrated, and it was concluded that the acetoxy- and methoxy-groups are devoid of significant orientational preference, but that the hydroxy-group has slight preference for the equatorial disposition.[672]

Methods for the assignment of anomeric configuration to pyrimidine nucleosides[662b] and to 2-deoxy-ribofuranosyl nucleosides,[662c] based on n.m.r. data, have been described.

Techniques for the assignments of anomeric configuration by other methods have been developed. A micro-method for the configuration of glycosidic linkages used a glycosidase in combination with glucose oxidase.[673a] Enzymic methods for glycosyl fluorides[343] and for some nucleosides[673b] have also been described.

[669] G. Gatti, A. L. Segre, and C. Morandi, *J. Chem. Soc.* (*B*), 1967, 1203.
[670] F. Sweet and R. K. Brown, *Canad. J. Chem.*, 1967, **45**, 1007.
[671] J. L. Coke and M. C. Mourning, *J. Org. Chem.*, 1967, **32**, 4063.
[672] R. J. Ferrier and N. Prasad, *J. Chem. Soc.* (*C*), 1967, 1417.
[673a] J. H. Pazur and A. Cepure, *Carbohydrate Res.*, 1967, **5**, 359.
[673b] E. Lodemann and A. Wacker, *Z. Naturforsch.*, 1967, *B*, **22**, 42.

24
Other Physical Methods

I.r. Spectroscopy

No major developments have been reported on the application of i.r. spectroscopy but several papers have appeared on specific topics in this area; one observation of general interest has, however, emerged. Spectra were appreciably improved when measurements were carried out at low temperatures; not only was band definition increased but new bands appeared. Since the changes were continuous as the temperature of measurement was reduced they were dependent not upon phase changes but upon restrictions of rotation about bonds. The spectra of trehalose dihydrate measured at 25 and $-160°$ in Nujol were illustrated for the range 200–2000 cm.$^{-1}$ [24] The spectra of a wide selection of *N*-acylglycosylamines, 1,1-bisacylamido-1-deoxyalditols and esterified derivatives have been presented and discussed at length, and the values of various correlations between structural and spectral features were assessed.[420] Phenylhydrazones of D-galactose, D-glucose, and D-mannose studied in the solid state were shown to have cyclic structures contrary to conclusions based on chemical methods, which were shown to be unreliable for such purposes.[401] Russian workers have examined the spectra of a variety of cyclic carbohydrate compounds in the region 700–950 cm.$^{-1}$ and correlations have been made between the positions of absorption bands and the orientation of C—H bonds.[674] It has been shown that the 845 cm.$^{-1}$ band of cyclopentane epoxides is a C—O stretching absorption, and not, as has sometimes been thought, derived from a C—H rocking vibration.[570]

I.r. spectroscopy has been applied to other carbohydrate derivatives as follows: sulphate esters,[293] nitrate esters,[335] carbonates,[259] and solid hydroxylated compounds.[152]

Mass Spectrometry

A review on the mass spectrometry of various natural products dated 1966 has appeared during 1967.[675] Interest in the technique is increasing and the spectra of a variety of compounds have been reported. In the main,

[674] V. P. Komar, R. G. Zhbankov, and A. M. Prima, *Zhur. strukt. Khim.*, 1967, **8**, 252.
[675] K. Bieman, *Prog. Chem. Org. Nat. Prod.* 1966, **24**, 1.

simple derivatives of monosaccharides have been investigated but the method need not be confined to these as is well illustrated by its use with a tetrasaccharide antibiotic.[607]

Hydrolysis of methylated polysaccharides followed by reduction and acetylation gave partially methylated alditol acetates of hexoses, pentoses, and deoxyhexoses which have been subjected to examination. Stereoisomers with the same pattern of substitution gave almost identical spectra, but differently substituted derivatives afforded characteristically different patterns, so mass spectrometry should provide an efficient means for identifying partially methylated sugars especially when used in conjunction with g.l.c.[676] Russian workers are developing a closely related approach to this analytical problem but have been using deuteriomethyl derivatives rather than acetyl esters to complete the substitution.[162–165] Heyns and his co-workers have approached the problem of characterising partially methylated 2-acetamido-2-deoxy-D-galactoses and their methyl glycosides in a similar fashion, but again have used acetates to complete the substitution. The fragmentation patterns of the various compounds studied were discussed.[677]

In a more specific application the mass spectra of peracetylated derivatives of synthetic and natural 6,6′-diesters of trehalose containing acyl radicals varying in size from C_{22} to C_{90} have been investigated.[678] Other applications of mass spectrometry have been referred to.[57, 130, 133, 151, 162, 179, 180, 242, 607]

X-ray Crystallography

Sufficient crystallographic analyses of carbohydrates have now been published for a beginning to be made to correlating the findings, and Jeffrey and his co-workers have considered C—O bond lengths in the neighbourhood of the anomeric centre. The exocyclic bond is, on average, significantly shorter than the endocyclic bond, and for pyranose molecules in which the C-1 to 1-O bond is axial, the 5-O to 1-C bond lengths are appreciably shorter than C-5 to 5-O lengths. This last observation correlates with the existence of the anomeric effect but the basis of this correlation has still to be explained.[679]

In a full structural analysis α-D-glucoisosaccharinic acid has been shown to be 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid,[538] and, not surprisingly, D-glucosamine hydrochloride was shown to exist in the chair conformation in the solid state.[680] Other applications of *X*-ray crystallography have been mentioned.[480, 587]

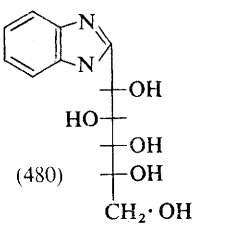
[676] H. Björndal, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1967, **5**, 433.
[677] K. Heyns, G. Kiessling, and D. Müller, *Carbohydrate Res.*, 1967, **4**, 452.
[678] A. Adam, M. Senn, E. Vilkas, and E. Lederer, *European J. Biochem.*, 1967, **2**, 460.
[679] H. M. Berman, S. S. C. Chu, and G. A. Jeffrey, *Science*, 1967, **157**, 1576.
[680] G. N. Ramachandran, R. Chandrasekaran, and K. S. Chandrasekaran, *Biochim. Biophys. Acta*, 1967, **148**, 317.

E.s.r. Spectroscopy

A few carbohydrate systems in which free radicals are formed have been examined by e.s.r. techniques, but it does not appear that this branch of spectroscopy will be of much general value in the field. Some applications reported during 1967 have already been mentioned.[27-29]

25
Polarimetry

Slowly, carbohydrate chemists are turning from monochromatic polarimetry to optical rotatory dispersion (and to a lesser extent circular dichroism) with beneficial effects, but considerable progress has still to be made before a proper understanding of the relationship of dispersion curves and structural features is established. Nevertheless, in many instances, polarimetry can be invaluable in structural (particularly configurational) analysis. For example, it has been shown that configuration can be assigned at positions 2, 3, 4, and 5 of aldoses from examination of the o.r.d. curves of appropriate derivatives. Benzimidazole, quinoxaline, flavazole, and anhydro-osazone derivatives [(480)–(483) for D-glucose] are



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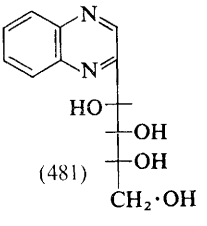

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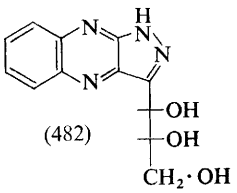
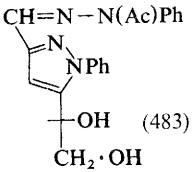


(482)



(483)

of value for this purpose, and, with each, the asymmetry at the carbon atom adjacent to the chromophore may be definable from the o.r.d. curve. U.v. spectra and o.r.d. curves of several carbohydrate compounds of this type were given, but the study was not sufficiently comprehensive to allow definite generalisations to be put forward.[681]

In related fashion configuration at C-2 may be determined for the o.r.d. curves of 1-deoxy-1-nitroalditols, and the method was applicable to nitro-alcohols formed by nitromethane additions to aldehydic compounds.

[681] W. S. Chilton and R. C. Krahn, *J. Amer. Chem. Soc.*, 1967, **89**, 4129.

Both the sign of the Cotton effect observed at 310 mμ and the ellipticity of the circular dichroism curve at this wavelength could be related to the configuration at the α-carbon.[682a] *N*-Salicylidene derivatives of amino-sugars give anomalous o.r.d. curves[391] which have been used in the study of antibiotic compounds.[392] The circular dichroism of carbohydrate nitrates has already been mentioned.[336]

Rotations of the eight 1,6-anhydro-D-aldopyranoses and their acetates have been rationalised consistently by an empirical procedure[192] and the o.r.d. spectra of carbonyl oxidation products of the hydroxy-compounds have been considered on the basis of the Octant Rule.[190, 191] Conclusions reached on the conformations of the compounds were confirmed by detailed n.m.r. spectroscopic measurements.[191] O.r.d. studies on 2-deoxy-glycosides[150] and on purine and pyrimidine nucleosides[682b] have also been reported.

The previously reported anomalous optical rotations of the anomeric 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-D-glucopyranoses have been further examined, and it has been shown that over the temperature range 20–60° and in chloroform, benzene, pyridine, acetone, and methanol, the β-isomer was consistently more dextrorotatory than its anomer.[683] Furthermore, in chloroform solution at 25° they retained their anomalous relationship over the wavelength range 300–500 mμ. Together with some unsaturated glycopyranosyl esters, these acetates are believed to be the only compounds known with simple aglycones which show these irregular rotational features. It was observed that the rotational anomaly was greatest in solvents of low polarity which helped to substantiate the hypothesis that the effect results from a constraint imposed by the *o*-nitro-group, generating an asymmetric chromophore, the rotational contribution of which competes with that of the anomeric centre. Such constraint was believed to result from dipolar interactions and would be expected to decrease in solvents of high polarity and also at elevated temperatures.

It has been shown that rotations of arabinose ethers can indicate the ring-size and hence, in some cases, position of substitution.[158]

Complex results of an inconclusive nature were obtained when specific rotations of thirteen carbohydrate derivatives were measured in *p*-dioxan–water mixtures,[684] and comments have been made on the comparison of calculated and observed optical rotations of trehalose derivatives.[685]

[682a] C. Satoh, A. Kiyomoto, and T. Okuda, *Carbohydrate Res.*, 1967, **5**, 140.
[682b] D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 1138.
[683] S. Guberman and D. Horton, *J. Org. Chem.*, 1967, **32**, 294.
[684] A. J. Hannaford, *Carbohydrate Res.*, 1967, **3**, 295.
[685] G. C. Birch and N. D. Cowell, *Carbohydrate Res.*, 1967, **5**, 232.

26
Separatory and Analytical Methods

In this Section the separatory techniques which are applied for the purification, characterisation, or quantitative determination of carbohydrates are dealt with, followed by direct analytical procedures.

Chromatographic and Related Methods

Gas–liquid Chromatography.—Several references to g.l.c. are made in Section 4. Two papers have described the use of pertrifluoroacetyl derivatives for the separation of a wide variety of cyclitol and sugar derivatives,[686, 687] including amino-sugars and disaccharides.[687] It was claimed that nanogram quantitities may be detected using this technique.[686]

Use of an inert standard, such as terphenyl or triphenylethane has been suggested[688] in the quantitative analysis of α- and β-D-glucose as their TMS derivatives; the accuracy was claimed to be ±1%. A method based on g.l.c. techniques has been developed for studying the mutarotation of sugars.[18]

A procedure has been described for the simultaneous analysis of all the monosaccharides which usually occur in glycoproteins.[689] The method, which has been applied to both model compounds and to glycoproteins, involved methanolysis, *N*-acetylation of the amino-sugars, and analysis of the resulting mixtures by g.l.c. of the TMS derivatives. A similar technique has been developed for the analysis of the components of mucopolysaccharides. The amino-groups were protected by *N*-ethoxycarbonylation followed by conversion into the TMS derivatives.[690] The method was satisfactory, although the different anomeric forms of each sugar gave rise to separate peaks. This was overcome by conversion of the liberated free sugars into the corresponding alditol acetates and the method has been applied to the analysis of complex soil polysaccharides.[691] Three other papers have described the use of g.l.c. methods for the separation of alditol acetates. Partially methylated monosaccharides from methylated polysaccharide hydrolysates have been reduced, acetylated, and then separated

[686] Z. Tamura and T. Imanari, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 246.
[687] T. Ueno, N. Kurihara, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 1189.
[688] Y. Halpern, Y. Houminer, and S. Patai, *Analyst*, 1967, **92**, 714.
[689] J. R. Clamp, G. Dawson, and L. Hough, *Biochim. Biophys. Acta*, 1967, **148**, 342.
[690] M. D. G. Oates and J. Schrager, *J. Chromatog.*, 1967, **28**, 232.
[691] J. M. Oades, *J. Chromatog.*, 1967, **28**, 246.

by g.l.c.,[692] the carbohydrate components of wood pulp have been determined by hydrolysis, borohydride reduction, and analysis of the alditol mixture by g.l.c. of their peracetates,[693] and the mixtures of polyhydroxy-compounds that resulted from the hydrogenolysis of carbohydrates have been analysed by g.l.c. as their peracetates.[694] Similarly, the polyols in plant tissues have been analysed, by first removing the reducing sugars by treatment with barium hydroxide, deionisation, and conversion of the polyols into their TMS derivatives followed by g.l.c.[695]

Lance and Jones[161] have noted that acetylated nitriles of free sugars were highly suited for the purposes of g.l.c. and were preferred to the acetylated diethyl dithioacetals which did not show discrete signals on the chromatograms.

Methyl glycosides of aldoses, ketoses, and hexuronic acids have been examined by g.l.c. of their TMS ethers,[696] and *N*-arylglucosylamines have been separated and identified by analysis of their corresponding derivatives.[418] G.l.c. data for the TMS ethers of some less common disaccharides obtained by partial hydrolysis of algal polysaccharides have been recorded,[697] and ascorbic acid can be determined by a method based on g.l.c. of its ether.[698]

Pyrolysis-gas chromatography has been used for the characterisation of several carbohydrate-containing antibiotics.[608]

Column Adsorption Chromatography.—Two column chromatography procedures have been claimed to have improved resolving powers. The first, 'short-column chromatography', used t.l.c. adsorbents in columns, and had the particular advantage of allowing the same separation on these columns as were achieved on analytical plates.[699] The second utilised dry columns and was rapid and efficient especially for coloured compounds or those which fluoresce under u.v. light. Nylon tubing, which has the advantage of being readily cut in sections after the development, was recommended in place of glass, and adsorbents containing fluorescent indicators were advocated and their preparation was described in detail.[700]

Paper and Thin-layer Chromatography.—The value of heavy chromatographic papers (particularly Whatman's seed paper and Whatman's No. 17) has been assessed for use in the separation of relatively large quantities of carbohydrates. These papers can be used to resolve easily separable mixtures at concentrations of *ca.* 2 g. and 0·6 g. per sheet, respectively; D-glucose and D-fructose were resolved when about half these quantities were applied. A simple nondestructive method was described for detecting

[692] H. Björndal, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1967, **21**, 1801.
[693] E. P. Crowell and B. B. Burnett, *Analyt. Chem.*, 1967, **39**, 121.
[694] G. Van Ling, C. Ruijterman, and J. C. Vlugter, *Carbohydrate Res.*, 1967, **4**, 380.
[695] N. V. Riggs and F. M. Strong, *Analyt. Biochem.*, 1967, **19**, 351.
[696] M. Tomoda, *J. Pharm. Soc., Japan*, 1967, **87**, 1057.
[697] E. Percival, *Carbohydrate Res.*, 1967, **4**, 441.
[698] M. Vecchi and K. Kaiser, *J. Chromatog.*, 1967, **26**, 22.
[699] B. J. Hunt and W. Rigby, *Chem. and Ind.*, 1967, 1868.
[700] B. Loev and M. M. Goodman, *Chem. and Ind.*, 1967, 2026.

the location of components on developed chromatograms, by which the thick paper was covered with a thin absorbent paper, pressed down in strips, and the transfer sprayed.[701]

The solvent system, butan-1-ol–benzene–formic acid–water (100 : 19 : 10 : 25, upper phase) has been found to separate glycoside anthocyanin pigments, when more than fifty other solvent systems would not.[702] The same system was also reported to be excellent for the resolution of mixtures of free sugars, giving compact spots after development; several sugars which are difficult to resolve in normal solvents were readily separated.[702] Systems for the paper chromatographic separation of mixtures of polyhydroxy-compounds obtained from the hydrogenolysis of carbohydrates have been described.[694]

It has been shown that at $-18°$, several monosaccharides give two spots on t.l.c. on cellulose. For example, D-fructose and D-mannose each gave two spots, whereas D-glucose only gave one.[703] It was suggested that this may be of use in the identification of minute amounts of some sugars. The preparative t.l.c. of pentoses, pent-2-uloses, and pent-3-uloses has been carried out on microcrystalline cellulose,[704] and the R_F values of ten free sugars, glucurono-γ-lactone, glucuronic and galacturonic acids, and 2-amino-2-deoxy-D-galactose and -glucose hydrochlorides on cellulose thin-layer chromatograms and in twenty-three solvents (some of which are particularly recommended) have been recorded.[705]

Conditions for the separation of aldoses from their corresponding alditols and aldono-lactones by t.l.c. on silica have been described.[706] The method was advocated for following the reduction of the lactones to aldoses and thence to alditols. Improved separations of sugars (compared with paper chromatography) were obtained when the silica-gel support was impregnated with inorganic salts, such as sodium mono- or di-hydrogen phosphate, and the procedure was recommended for the analysis of polysaccharide hydrolysates. Results of an investigation of a wide range of carbohydrates, and conditions are given.[707] A paper describing a very similar approach has appeared.[708] T.l.c. has also been employed in the separation of various methylated methyl glycosides.[709]

A modified form of t.l.c. which uses the inside conical surface of filter funnels has been found to be of particular value with compounds which tend to trail; it has been shown to be applicable to the separation of anomeric

[701] H. L. Frush, *J. Res. Nat. Bur. Stand., Sec. A*, 1967, **71**, 49.
[702] T. Fuleki and F. J. Francis, *J. Chromatog.*, 1967, **26**, 404.
[703] G. Avigad and S. Bauer, *Carbohydrate Res.*, 1967, **5**, 417.
[704] K. Linek, L. Kuniak, and B. Alince, *Chem. Zvesti.* 1967, **21**, 99 (*Chem. Abs.*, 1967, **67**, 32895*v*).
[705] M. Tomoda, *J. Pharm. Soc., Japan*, 1967, **87**, 207.
[706] J. Nemec, K. Kefurt, and J. Jarý, *J. Chromatog.*, 1967, **26**, 116.
[707] Yu. S. Ovodov, E. V. Evtushenko, V. E. Vaskovsky, R. G. Ovodova, and T. F. Solov'eva, *J. Chromatog.*, 1967, **26**, 111.
[708] A. Lombard, *J. Chromatog.*, 1967, **26**, 283.
[709] V. T. Bykov, A. A. Vaskovskaya, and V. E. Vaskovsky, *J. Chromatog.*, 1967, **30**, 643.

glycosides and other carbohydrate derivatives.[710] Circular t.l.c. has also been described,[711] and thick polyamide layers have been suggested for the rapid separation of sugars at loads higher than those generally used on t.l.c. plates.[712] An aid to preparative chromatography involves the transference of a thin layer of developed t.l.c. plates to an adhesive surface, and the detection of the separated components on this. By this procedure the compounds could be located on the main plate and then eluted.[713a] For u.v. absorbing compounds a detection method which employed u.v. transparent plates has been reported. After separations (two-dimensional if required) were complete, the plates were simply placed over photosensitive paper and illuminated with u.v. light.[713b]

Conditions for the separation of a wide range of nucleosides and their derivatives by t.l.c. have been described[714] and a large number of R_F values tabulated both for t.l.c. and paper chromatography.[715, 716]

The system *p*-anisidine–ethanol, followed by sodium periodate in acetone, has been described as a spray reagent for the detection and differentiation of carbohydrate compounds by their colour reactions on paper or t.l.c. The method was applied to polyols, pentoses, hexoses, their 2-deoxy-derivatives, sugar acids, and amino-sugars and their *N*-acetyl derivatives. The sensitivity was 1 to 20 μg., depending on the class of compound.[717]

A new chromatography spray reagent has been developed, based on the formation of 3,5-diacetyl-1,4-dihydrolutidine, from formaldehyde liberated on periodate oxidation of sugars.[718] The chromatogram was sprayed with periodic acid in the presence of pyridine, followed by acetylation in the presence of ammonium acetate and acetic acid. Hexoses and pentoses gave spots which were detectable in u.v. light (1 μg. sugar per cm.^{2}); amino-sugars and all other derivatives that gave formaldehyde on oxidation gave yellow spots on chromatograms with yellow-green fluorescence under u.v. light.

Improvements in the use of the aminoguanidine t.l.c. spray reagent which allow the detection of 0·1 μg. of aldoses and ketoses have been described. Fucose gave a specific colour reaction and could be identified by this means.[719] A t.l.c.-based method has been developed for the analysis of the sugars resulting from the hydrolysis of flavonoid glycosides.[720]

[710] K. Brendel, R. S. Steele, and E. A. Davidson, *J. Chromatog.*, 1967, **30**, 232.
[711] S. Chiba and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 255.
[712] J. P. Marais, *J. Chromatog.*, 1967, **27**, 321.
[713a] D. Horton and T. Tsuchiya, *Carbohydrate Res.*, 1967, **5**, 426.
[713b] B. Arreguin, *J. Chromatog.*, 1967, **26**, 527.
[714] G. Pataki and A. Niederwieser, *J. Chromatog.*, 1967, **29**, 126, 133.
[715] *J. Chromatog.*, 1967, **26**, D10, D11.
[716] K.-H. Scheit, *Biochim. Biophys. Acta*, 1967, **134**, 217.
[717] L. A. Veiga and E. L. Chandelier, *Analyt. Biochem.*, 1967, **20**, 419.
[718] J. B. Weiss and I. Smith, *Nature*, 1967, **215**, 638.
[719] P. M. Martins and Y. P. Dick, *J. Chromatog.*, 1967, **32**, 188.
[720] J. W. Mizelle, W. J. Dunlap, and S. H. Wender, *J. Chromatog.*, 1967, **28**, 427.

A procedure has been described for the quantitative determination of any sugar that can be separated by t.l.c. as its reduction product from other alditols, and has been applied, for example, to samples containing as little as 0·3 μg. of L-fucose. The sugar, with an internal standard (in this case, D-ribose), was reduced with sodium borotritiide and the resulting radioactive polyols were separated by t.l.c. and determined by scintillation counting[721] after extraction.

Ion-exchange Chromatography.—The applications of this technique to the separation of carbohydrates have been reviewed,[722] and details of automatic assemblies have been described.[723–725] Separations of a wide range of amino-sugars and amino-acids on Dowex-50 using pyridine–acetic acid buffers have been coupled with the use of an autoanalyser which employed a ninhydrin detector.[726, 727]

Strongly basic anion-exchange resins in the bisulphite form have been used for the separation of aldoses and ketoses and for the preparation of pure samples of D-glucose, D-galactose, and D-fructose from commercial products.[728] A rapid technique for the quantitative determination of carbohydrates has been developed based on the use of anion-exchange resins in the borate form; mixtures of mono-, di-, and tri-saccharides have been analysed as well as soluble hemicelluloses and glucose polymer-homologues.[729] The same form of resin has been used for the analysis of polyols in urine. Good separations of tetritols, pentitols, and hexitols were achieved; isomers with vicinal *trans*-diol groups in the Fischer projection formula were eluted before the *cis*-analogues.[730]

A new strong anion-exchange resin (Beckman Type 1-5) has been examined for use in sugar separations.[731] A synthetic mixture of five disaccharides and six monosaccharides was separable into its components. Mixtures of aldobiouronic acids have been separated on anion columns using sodium tetraborate as eluent.[537]

Electrophoresis.—The electrophoretic mobilities of many nucleosides and their derivatives have been collated.[715] A method for the separation of D-glucose, D-galactose, and D-fructose has been described.[732] (For other uses see refs. 469, 550, 553.)

[721] M. Murakami and R. J. Winzler, *J. Chromatog.*, 1967, **28**, 344.
[722] O. Samuelson, *Analyt. Chim. Acta*, 1967, **38**, 163.
[723] P. Jonsson and O. Samuelson, *J. Chromatog.*, 1967, **26**, 194.
[724] L.-I. Larsson and O. Samuelson, *Mikrochim. Acta*, 1967, 328.
[725] P. Jonsson and O. Samuelson, *Analyt. Chem.*, 1967, **39**, 1156.
[726] K. Brendel, R. S. Steele, R. W. Wheat, and E. A. Davidson, *Analyt. Biochem.*, 1967, **18**, 161.
[727] K. Brendel, N. O. Roszel, R. W. Wheat, and E. A. Davidson, *Analyt. Biochem.*, 1967, **18**, 147.
[728] B. Lindberg and K. N. Slessor, *Carbohydrate Res.*, 1967, **5**, 286.
[729] R. B. Kesler, *Analyt. Chem.*, 1967, **39**, 1416.
[730] N. Spencer, *J. Chromatog.*, 1967, **30**, 566.
[731] J. I. Ohms, J. Zec, J. V. Benson, jun., and J. A. Patterson, *Analyt. Biochem.*, 1967, **20**, 51.
[732] D. A. Kennedy, *Analyt. Biochem.*, 1967, **18**, 180.

Other Analytical Methods

Several methods for the analysis of D-glucose have appeared. One procedure [733] enables this sugar to be determined in nanomolecular quantities in the presence of relatively high concentrations of other sugars, such as D-mannose, D-fructose, and 2-deoxy-D-glucose. The method utilized a highly purified D-glucokinase (from *Aerobacter aerogenes*) coupled with D-glucose 6-phosphate dehydrogenase; as an example, D-glucose was determined with an error of only 2·5% in a 1 : 3300 mixture with D-mannose. Another method for D-glucose analysis was based on oxidation by periodate, in which the iodine freed from excess periodate was determined by detection of the product (4,5-di-iodide) from its reaction with fluoroescein. The method was claimed to be useful for concentrations of the sugar between 0·5 and 1·5 μg. per ml.[734a] It would seem to be of value for this method to be further explored for general use in periodate-oxidation analysis. A micro-method for the determination of periodate, based on polarographic techniques, has been developed. The procedure was used successfully for the analysis of less than one μmole of sugar.[734b]

A titrimetric method for determining glycols based on their stoicheiometric reduction of gold chloride in alkaline media has been described,[735] and a technique has been developed for the determination of primary and secondary hydroxy- or amino-groups in carbohydrates. In it, the 3,5-dinitrobenzoyl derivatives were prepared and colours formed by them in acetone solution on the addition of ammonia were examined.[736] Methods have also been reported for the analysis of D-glucose in industrial D-glucose syrup,[737] body-fluids,[738] and blood or plasma.[739]

A method for the analysis of D-mannose in the presence of other sugars has been developed and was believed to be the first based on the use of plant lectins.[740] The procedure depends on the amount by which the turbidity formed on interaction of glycogen with concanavalin A is inhibited specifically by D-mannose. The range of concentrations covered by the method is 300–750 μg./ml.; corrections can be made for the presence of D-glucose. An isotopic-dilution method has been developed for the assay of free sugars in mammalian cells.[741] Methods have also been developed for the determination of D-galactose in biological fluids,[742] of D-ribose and

[733] M. Y. Kamel, R. R. Hart, and R. L. Anderson, *Analyt. Biochem.*, 1967, **18**, 270.
[734a] D. E. Braun and W. H. Wadman, *Analyt. Chem.*, 1967, **39**, 840.
[734b] W. G. Breck, R. D. Corlett, and G. W. Hay, *Chem. Comm.*, 1967, 604.
[735] V. N. P. Srivastava and O. C. Saxena, *Microchem. J.*, 1967, **12**, 435.
[736] G. R. Umbreit and R. L. Houtman, *J. Pharm. Sci.*, 1967, **56**, 349.
[737] H. El Khadem, Z. M. El-Shafei, and H. S. Hekal, *Carbohydrate Res.*, 1967, **4**, 185.
[738] M. Gros and M. Smrekar, *Clinica Chim. Acta*, 1967, **17**, 518.
[739] J. D. Pryce, *Analyst*, 1967, **22**, 198.
[740] R. D. Poretz and I. J. Goldstein, *Carbohydrate Res.*, 1967, **4**, 471.
[741] L. Shen and V. Ginsburg, *Arch. Biochem. Biophys.*, 1967, **122**, 474.
[742] V. H. Förster and M. Halsbeck, *Z. Klinische Chem.*, 1967, **5**, 198.

its 2-deoxy-derivative,[42a] of 2-deoxy-D-ribose in DNA,[42b] of D-xylose,[743] and of D-fructose.[744, 745]

Modifications of the cysteine–sulphuric acid method for the analysis of hexoses have also been made to increase its sensitivity.[746] The conditions for the determination of small amounts of hexoses (*ca.* 10 μg.) by iodometry have been established; it was shown that iodide concentration is critical.[747]

Two titrimetric methods for the determination of free sugars based on their oxidation with vanadium(V) or cerium(IV) have been described; the latter was preferable since complete oxidation to carbon dioxide was involved.[748] In the vanadium case, 'bright sunlight' was required and is therefore hardly recommendable for general use.

The ferricyanide test has been applied to seventeen sugars apart from D-glucose for which it was initially developed. All reducing sugars gave a positive test, all nonreducing ones did not; deoxy- and substituted sugars gave a less intense reaction than did the parent sugars. The test was assessed as being useful for a wide variety of free sugars.[749]

Procedures for the determination of hexuronic acids and hexoses independently in mixtures of each other, based on the carbazole reaction,[750] and one for the analysis of hexuronic acids and ketoses in the presence of aldoses [751] have been recorded. A new highly sensitive and reproducible method for the determination of free sugars and α-glycol compounds generally has been described, based on the use of 3-methyl-2(3*H*)-benzothiazolone.[752] Comparison with other colorimetric methods showed it to be superior.

A modification of the method of determining hexoses, pentoses, and uronic acids and their glycosides by reaction with sulphuric acid has been developed, the concentration of the acid used being critical. At concentrations of *ca.* 50 μg./ml., 0·25 ml. can be analysed to within 2·5%.[753]

The specific analysis of uronides is of importance in biochemical work and developments have been reported; modifications of the carbazole colorimetric method for determining hexuronides and hexosamineuronides, in which the concentrations, reaction temperatures, and times were varied, allowed the reaction to be used to distinguish between various individual compounds.[754]

[743] F. K. Stevenson, P. W. Kent, and D. Fisher, *Chem. and Ind.*, 1967, 703.
[744] J. S. Davis and J. E. Gander, *Analyt. Biochem.*, 1967, **19**, 72.
[745] E. Van Handel, *Analyt. Biochem.*, 1967, **19**, 193.
[746] Z. Dische and A. Danilchenko, *Analyt. Biochem.*, 1967, **21**, 119.
[747] C. Hatanaka, *J. Agric. Chem. Soc., Japan*, 1967, **41**, 448.
[748] G. R. Bhansali, D. L. Mathur, and S. P. Rao, *Indian J. Chem.*, 1967, **5**, 454.
[749] G. Guinn, *J. Chromatog.*, 1967, **30**, 178.
[750] J. T. Galambos, *Analyt. Biochem.*, 1967, **19**, 119, 133.
[751] Y. Milner and G. Avigad, *Carbohydrate Res.*, 1967, **4**, 359.
[752] E. Sawicki, R. Schumacher, and C. R. Engel, *Microchem. J.*, 1967, **12**, 377.
[753] R. W. Scott, W. E. Moore, M. J. Effland, and M. A. Millett, *Analyt. Biochem.*, 1967, **21**, 68.
[754] Z. Dische and C. Rothschild, *Analyt. Biochem.*, 1967, **21**, 125.

It has been emphasised that there are disadvantages in the use of acidic or enzymic hydrolyses of pregnanediol glucuronide which have been used for the liberation of the sterol prior to its determination in urine. These have now been overcome by pretreating the uronide with periodate at pH 3·5 which causes oxidation of the carbohydrate moiety and hydrolysis of the residual fragments. The conjugate can then be determined by analysing, with standard procedures, for the sterol.[755] A further method has been developed for analysing for glucuronic acid conjugates and involved their separation on a resin chromatographic column and gas chromatography of standard volatile derivatives.[756]

The use of [^{14}C]acetic anhydride for the assay of hexosamines in hydrolysates from purified oligosaccharides, in particular for 2-amino-2-deoxy-D-galactose and -galactitol, has been described. The products were separated electrophoretically and the resultant strips counted.[757] Modifications in the Elson–Morgan [758] and the Good–Bessman [759] methods for the analysis of hexosamines have been reported, as has a method for the separation and determination of 2-amino-2-deoxy-D-glucose and -galactose in biological fluids.[760]

An automated procedure [761] and one dependent on the reduction of mercury(II) to mercury(I) and then a complexiметric titration [762] have been reported for the determination of ascorbic acid. These have been established for analyses of biological fluids, and were effective in the presence of, for example, D-glucose.

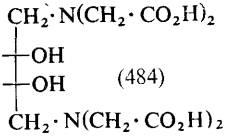
[755] A. P. Wade, *Biochem. J.*, 1967, **103**, 19c.
[756] J. B. Knaak, J. M. Eldridge, and L. J. Sullivan, *J. Agric. Food Chem.*, 1967, **15**, 605.
[757] D. M. Carlson, *Analyt. Biochem.*, 1967, **20**, 195.
[758] J. Ludowieg and J. D. Benmaman, *Analyt. Chem.*, 1967, **19**, 80.
[759] D. Glowacka, T. Kopacz-Jodczyk, and J. Popowicz, *Analyt. Chem.*, 1967, **19**, 1.
[760] R. M. Nair and P. A. Kurup, *Indian J. Biochem.*, 1967, **4**, 117.
[761] B. L. Nessett, B. L. Windsor, R. R. Humphrey, and M. R. Callantine, *Analyt. Biochem.*, 1967, **19**, 89.
[762] S. Baczyk and O. Kachelska, *Mikrochim. Acta*, 1967, 143.

27
Alditols*

The root bark of the common Indian shrub, *Clerodendron serratum*, has been shown to contain about 10% of D-mannitol.[763, 764]

The action of mixtures of trifluoracetic acid and acetic anhydride on 1,3:2,5:4,6-tri-*O*-methylene-D-mannitol has been investigated.[211] The synthesis of the following acetals has been described: 3,4-*O*-isopropylidene-D-mannitol,[258] 1,3:4,6-di-*O*-chloroethylidene-galactitol,[210] and the 2,3:4,5-di-*O*-isopropylidene acetals of D-arabinitol and xylitol.[209]

1-Amino-1-deoxy-derivatives of D-*glycero*-D-*gulo*-, D-*glycero*-D-*galacto*-, and D-*glycero*-L-*manno*-heptitols have been prepared.[370] The synthesis of the complexone (484) has been described.[765]



(484)

New syntheses of the 1-, 2-, and 3-*O*-methyl-D-mannitols[157] and of 1-*O*-benzyl-L-glycerol[167] have been developed. TMS ethers of xylitol and some of its anhydro-derivatives have been characterised.[178] A detailed kinetic study has been made on the acid-catalysed dehydration of tetritols and pentitols.[199]

Several papers have appeared on 1,6-dibromo-1,6-dideoxy- and 1,6-dimethanesulphonyl-hexitols with particular emphasis on the formation of epoxides from them.[188, 189, 351]

The oxidation of D-sorbitol,[655] of 1-deoxy-D-galactitol,[653] and of D-galactose diethyl dithioacetal and dimethyl acetal[653] by *Acetobacter suboxydans* has been investigated.

G.l.c. analysis of alditols, or their partially methylated derivatives, as their peracetates,[692-694] or as their TMS ethers,[695] has been described. A method has been developed for the separation of polyols using an anion-exchange resin in the borate form.[730]

[763] S. C. L. Verma, V. P. Garg, and S. S. Gupta, *Current Sci.*, 1967, **36**, 126.
[764] V. P. Garg and S. C. L. Verma, *J. Pharm. Sci.*, 1967, **56**, 639.
[765] I. A. Seliverstova, O. I. Samoilova, N. M. Dyatlova, and V. G. Yashunskii, *Zhur. obshchei Khim.*, 1967, **37**, 2643.

* For the terms of reference used for this Section, see the Introduction.

Part II

MACROMOLECULES

1
Introduction

The primary objective in preparing this Report has been to summarise information, published during 1967, on the chemistry of macromolecules that contain carbohydrate; in a few cases, literature published in 1968 has been included. The Report is concerned mainly with structural studies of such molecules, and, to a lesser extent, with their physical and biological properties and biosynthesis. The amount of background information included is minimal, and certain topics, e.g. studies of the structure and specificity of lysozyme, either have been omitted, or, have been cited briefly with a list of appropriate references.

Time has prevented the abstraction of all material, especially certain foreign journals and patents, published during the time period mentioned above. While care has been taken to be comprehensive, notification of any omissions would be welcomed so that relevant material can be included in future volumes in this series.

2
General Methods

Analysis*

Modifications of the primary and secondary cysteine–sulphuric acid reactions of hexoses have been described [1] which give greater sensitivity and better reproducibility. Two modifications of the carbazole–sulphuric acid reaction of hexuronic acids permit the differentiation of hexosamino-hexuronides.[2] An ultraspecific enzymic method has been reported [3] for the determination of D-glucose in the presence of a 3000-fold excess of D-mannose.

Several methods have been reported for the analysis of sugars in polysaccharide hydrolysates by g.l.c. The method has been used to determine the anomeric composition of aqueous solutions of simple sugars [4] and the anomeric configuration of D-glucose liberated by enzymic hydrolysis of glucans and glucosides.[5] The monosaccharides commonly present in glycoproteins have been identified and estimated simultaneously after methanolysis, *N*-acetylation, and conversion to TMS derivatives.[6] The simultaneous determination of L-fucose, D-mannose, D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose as their TMS derivatives after prior carboxyethylation of amino-sugars was described.[7] A convenient method for the analysis of sugars in plant polysaccharides employed hydrolysis with 2N-trifluoroacetic acid, which was subsequently removed by evaporation, followed by sequential reduction with borohydride, acetylation, and g.l.c. analysis of the acetates.[8] G.l.c. was convenient for the rapid analysis of methylated polysaccharides, particularly when comparisons were required within a uniform group of polysaccharides such as certain plant gums.[9]

[1] Z. Dische and A. Danilchenko, *Analyt. Biochem.*, 1967, **21**, 119.
[2] Z. Dische and C. Rothschild, *Analyt. Biochem.*, 1967, **21**, 125.
[3] M. Y. Kamel, R. R. Hart, and R. L. Anderson, *Analyt. Biochem.*, 1967, **18**, 270.
[4] R. Bentley and N. Botlock, *Analyt. Biochem.*, 1967, **20**, 312.
[5] F. W. Parrish and E. T. Reese, *Carbohydrate Res.*, 1967, **3**, 424.
[6] J. R. Clamp, G. Dawson, and L. Hough, *Biochim. Biophys. Acta*, 1967, **148**, 342.
[7] M. D. G. Oates and J. Schrager, *J. Chromatog.*, 1967, **28**, 232.
[8] P. Albersheim, D. J. Nevins, P. D. English, and A. Karr, *Carbohydrate Res.*, 1967, **5**, 340.
[9] M. Kaplan and A. M. Stephen, *Tetrahedron*, 1967, **23**, 193.

* See also Part I, Sections 24 and 26.

Mass spectrometric analysis of certain sugar derivatives has provided an additional parameter in the identification of sugars.[10, 11] For example, the TMS derivatives of diastereoisomeric aldonolactones were differentiated by mass spectrometry.[10] The combined use of g.l.c. and mass spectrometry permitted the identification of microgram quantities of certain sugar derivatives.

Further work has been reported on the automated analysis of monosaccharides by partition chromatography on ion-exchange resins.[12] The alditols glycerol, threitol, xylitol, arabitol, ribitol, gulitol, galactitol, and mannitol were separated by ion-exchange chromatography on De-Acidite resin FF in the borate form.[13]

Structural Methods

Possible methods for obtaining, from simple kinetic data, information on the distribution of monosaccharide residues in heteropolysaccharides have been investigated.[14] Equations that related the combined yield and average molecular weight of all fragments containing only one kind of monomer unit to the overall degree of scission were derived for heteropolysaccharides in which the monosaccharides were arranged either uniformly or according to a simple statistical model. For a given degree of scission these quantities were markedly dependent upon the asymmetry in the distribution of monosaccharide residues along the polymer chain.

Specific methods for assaying isosaccharinic, metasaccharinic, and saccharinic acids on a microgram scale have permitted monitoring of the alkali-mediated peeling of oligo- and poly-saccharides.[15] The assays were based on specific colorimetric or enzymic reactions of products obtained by oxidation of the various saccharinic acids with periodate.

Selective cleavage of polysaccharide chains at the linkage involving C-4 of residues of uronic acid has been investigated in a heterogeneous reaction of 2-hydroxyethylalginate with methanolic sodium methoxide.[16] Fragmentation of the polysaccharide chain was incomplete and the rate of reaction was not diagnostic of the stereochemistry of the glycopyranuronosyl residue. The unsaturated acidic product underwent further reaction. Selective cleavage of hexuronidic linkages has been achieved by conversion of the uronic acid residue to an amide followed by a Hofmann degradation of the amide to a 5-amino-5-deoxy-pentopyranose which was hydrolysed

[10] G. Petersson, O. Samuelson, K. A. Anjou, and E. V. Sydow, *Acta Chem. Scand.*, 1967, **21**, 1251.
[11] G. Bjorndal, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1967, **5**, 433.
[12] P. Jonsson and O. Samuelson, *J. Chromatog.*, 1967, **26**, 194.
[13] N. Spencer, *J. Chromatog.*, 1967, **30**, 566.
[14] T. J. Painter, *J. Chem. Soc.* (*C*), 1967, 922.
[15] S. A. Barker, A. R. Law, P. J. Somers, and M. Stacey, *Carbohydrate Res.*, 1967, **3**, 435.
[16] C. W. McCleary, D. A. Rees, J. W. B. Samuel, and I. W. Steele, *Carbohydrate Res.*, 1967, **5**, 492.

by mild acid conditions.[17] In this way, glycuronosidic bonds could be cleaved under conditions where glycosidic bonds were stable.

Polyaldehydes produced by periodate-oxidation of polysaccharides gave bisulphite addition compounds (polyhydroxysulphonic acids) which formed precipitates on addition of long-chain, quarternary ammonium compounds.[18] The solubility properties of the precipitates were typical of those of quaternary ammonium salts of other polyanions. Oxidizable and non-oxidizable oligo- or poly-saccharides, glycoproteins, etc. have been separated by paper electrophoresis in bisulphite buffer, pH 5·6 or pH 2·0, or by selective precipitation of the polyhydroxysulphonic acid derivative of the oxidized material with quaternary ammonium compounds.

The production of formic acid by reduction of carbon dioxide with borohydride might impair the results of quantitative studies of polysaccharide degradation[19] (*e.g.* by sequential oxidation with periodate, reduction with borohydride, and subsequent mild hydrolysis with acid, in the Smith degradation). Excess formate might arise from the deliberate destruction of borohydride with carbon dioxide, or from atmospheric contamination, and from borohydride reduction in solutions buffered with carbon dioxide or with hydrogen carbonate.

Chemical coupling of proteins to polysaccharides has provided biologically active, water-insoluble polymers. Biologically active peptides and proteins (*e.g.* chymotrypsin) have been coupled to starch, cellulose, and Sephadex and Agarose gels by means of cyanogen halides.[20, 21] At room temperature the products were very stable over a wide range of pH. Reaction of copolymers of maleic anhydride and ethylene with trypsin, chymotrypsin, and kallikrein gave water-insoluble, biologically active resins which were especially useful for isolating low molecular weight natural inhibitors of the enzymes cited above.[22] Coupling of a protein antigen to diazotised *p*-aminophenylbutyrylaminoethyl cellulose gave a product which bound specifically antibody,[23] and highly purified antibody could be recovered from the complex by treatment with glycine buffer, pH 2·4. Other immunosorbents have been prepared by chemical coupling of antibodies to derivatives of Sephadex,[24] by polymerisation of antigens or antibodies by reaction with ethylchloroformate at pH 5,[25] and by reaction of bromacetyl-cellulose with peptide haptens or proteins.[26]

[17] N. K. Kochetkov, O. S. Chizhov, and A. F. Sviridov, *Carbohydrate Res.*, 1967, **4**, 362,
[18] J. E. Scott, *Chem. and Ind.*, 1967, 953.
[19] F. Eisenburg, jun. and A. H. Bolden, *Carbohydrate Res.*, 1967, **5**, 349.
[20] R. Axén, J. Porath, and S. Ernback, *Nature*, 1967, **214**, 1302.
[21] J. Porath, R. Axén, and S. Ernback, *Nature*, 1967, **215**, 1491.
[22] H. Fritz, H. Schult, M. Hutzel, M. Wiedemann, and E. Werle, *Z. physiol. Chem.*, 1967, **348**, 308.
[23] M. M. Behrens, J. K. Inman, and W. E. Vannier, *Arch. Biochem. Biophys.*, 1967, **119**, 411.
[24] L. Wide, R. Axén, and J. Porath, *Immunochemistry*, 1967, **4**, 381.
[25] S. Avrameas and T. Ternynck, *J. Biol. Chem.*, 1967, **242**, 1651.
[26] J. B. Robbins, J. Haimovich, and M. Sela, *Immunochemistry*, 1967, **4**, 11.

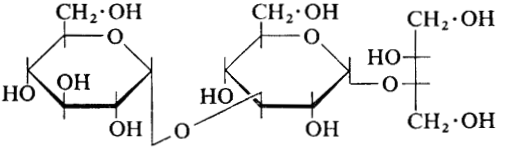
Specific Interactions of Carbohydrates with Concanavalin A

Concanavalin A, the phytohaemagglutinin of the jack bean, reacts specifically to form a precipitate with a restricted group of branched polysaccharides. Quantitative studies of the effects of pH, salt concentration, temperature, time, and volume of reagents on the interaction of concanavalin A with a dextran showed[27] that the concanavalin A–polysaccharide interaction displayed many of the characteristics of an antibody–antigen system. Concanavalin A was conveniently purified by methods based on its specific adsorption on to Sephadex gels and subsequent displacement with solutions of D-glucose.[28] Some physical and chemical properties of the purified protein have been reported.[29, 30]

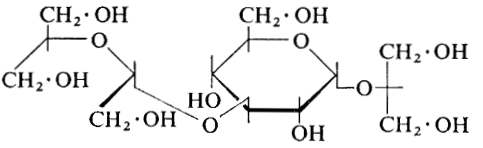
Inhibition studies of the concanavalin A–dextran interaction, using as inhibitors oligosaccharides of the maltose, isomaltose and methyl α-maltoside series, amino- and acetamido-deoxy-sugars, and oligosaccharides containing more than one type of linkage, essentially confirmed that interaction occurred between concanavalin A and the terminal, non-reducing, ends of α-linked glucans and mannans.[31] The combining sites of the concanavalin A molecule were complementary to the hydroxy-groups at C-3, C-4, and C-6 of α-D-glucopyranosyl and α-D-mannopyranosyl units.[32] Any modification of the C-6-hydroxy-group of methyl α-D-glucopyranoside virtually eliminated the capacity of the resultant derivative to bind to the protein. The best direct evidence for the interaction of concanavalin A with saccharide chain ends was given by inhibition studies with nigerosyl erythritol (1) and its derivatives produced by sequential oxidation with periodate and reduction with borohydride (2) and subsequent mild hydrolysis with acid (3).[31] Compounds (1) and (3) both inhibited the concanavalin A–dextran interaction whereas (2), in which the glucosyl residue is substituted at C-3, was not an inhibitor. Inhibition studies in this system might be of general use in the structural analysis of oligosaccharides.

Specific terminal, nonreducing sugars, however, were not the sole moieties capable of interacting with concanavalin A. The protein interacted with the hydroxy-groups at C-3, C-4, and C-6 of the *reducing* D-glucopyranosyl residue of sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) and 2-*O*-β-D-galactopyranosyl-D-glucose.[33a] D-Galactose, its α- and β-glycosides, and sophoritol did not act as inhibitors in the concanavalin–dextran reaction.[33a] The protein also interacted to form a precipitate with bovine serum albumin containing multiple *p*-phenylazo-β-sophorosyl residues.

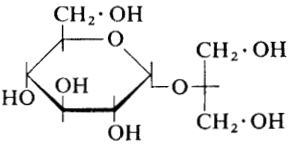
[27] L. L. So and I. J. Goldstein, *J. Biol. Chem.*, 1967, **242**, 1617.
[28] B. B. L. Agrawal and I. J. Goldstein, *Biochim. Biophys. Acta*, 1967, **147**, 262.
[29] B. B. L. Agrawal and I. J. Goldstein, *Biochim. Biophys. Acta*, 1967, **133**, 376.
[30] M. O. J. Olson and I. E. Liener, *Biochemistry*, 1967, **6**, 105.
[31] E. E. Smith and I. J. Goldstein, *Arch. Biochem. Biophys.*, 1967, **121**, 88.
[32] L. L. So and I. J. Goldstein, *J. Immunol.*, 1967, **99**, 158.
[33a] I. J. Goldstein, R. N. Iyer, E. E. Smith, and L. L. So, *Biochemistry*, 1967, **6**, 2373.



(1)



(2)



(3)

More recently it was shown [33b] that concanavalin reacted with polyelectrolytes (*e.g.* fucoidan, RNA, heparin, and certain bacterial lipopolysaccharides) that lacked terminal glucopyranosyl or mannopyranosyl residues. The introduction of polar residues such as acetate, formate, and phosphate into glycogen enhanced the precipitation with concanavalin A, whereas the opposite effect was noted on incorporation of methyl groups. Complex formation between polyelectrolyte and the protein was partially inhibited by salt and neutral sugars and it was suggested that hydrogen-bonding and electrostatic forces were involved in complex formation.

[33b] R. J. Doyle, E. E. Woodside, and C. W. Fishel, *Biochem. J.*, 1968, **106**, 35.

3
Plant Polysaccharides

The chemistry and biochemistry of pectic substances were the subjects of a recent review.[34]

It has been shown [35] that the stability constant of calcium pectate and the selectivity coefficient for calcium and potassium ions are good criteria of the distribution pattern of free carboxylic acid groups in pectin molecules.

Pectic substances extracted from lucerne (*Medicago sativa*) with ammonium oxalate contained approximately 75% of D-galacturonic acid together with L-rhamnose, L-arabinose, and D-galactose.[36] Partial acid hydrolysis and/or acetolysis liberated oligomers of D-galacturonic acid, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, higher oligosaccharides containing rhamnosyl and galactosyluronic acid residues, together with small amounts of the glucosyluronic acid-containing aldobiouronic acids 4-*O*-(β-D-glucopyranosyluronic acid)-L-fucose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

Comparative studies have been made [37] of the pectic substances in cured and uncured tobacco. Pectin from cured tobacco contained more than 90% of anhydrogalacturonic acid, mainly α(1→4)-linked. By contrast, the pectin from fresh leaf had a much lower anhydrouronic acid content (approximately 20%), but the acidic component was structurally similar to the pectic substance from cured tobacco. The pectin from fresh leaf readily liberated oligosaccharides containing neutral and acidic monosaccharides on partial hydrolysis. One of these was tentatively assigned the structure, 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose; a second oligosaccharide contained galacturonic acid, galactose, and rhamnose; and a third contained galacturonic acid and galactose.

D-Glucose and L-arabinose served as precursors of the pectic polysaccharides of sycamore callus-tissue.[38] D-Glucose was a precursor of both the neutral sugar portion of the pectin and of the galacturonan but not of the methyl groups, whereas L-arabinose could only act as a precursor of the neutral polysaccharides. A transferase present in mung beans (*Phaseolus*

[34] H. G. J. Worth, *Chem. Rev.*, 1967, **67**, 465.
[35] R. Kohn and I. Furda, *Coll. Czech. Chem. Comm.*, 1967, **32**, 4471.
[36] G. O. Aspinall, B. Gestetner, J. A. Molloy, and M. Uddin, *Israel J. Chem.*, 1967, **5**, 142P.
[37] E. J. Bourne, J. B. Pridham, and H. G. J. Worth, *Phytochemistry*, 1967, **6**, 423.
[38] R. W. Stoddart and D. H. Northcote, *Biochem. J.*, 1967, **105**, 45.

aureus) catalysed the transfer of the methyl group of S-adenosyl-L-methionine to galacturonan.[39] A similar transmethylation reaction was proposed[40] in the biosynthesis of the 4-*O*-methyl-D-glucuronosyl unit of hemicellulose. The disaccharide 2-*O*-(D-galactosyluronic acid)-L-rhamnose was present in sycamore whole pectin.[41] The differences in the pectins of callus, cambium, and fruit seemed to be characteristic of the nature of the growth and growth conditions of the cells.[41]

Two extracellular polygalacturonases of *Aspergillus niger* have been classified[42] as an endopolygalacturonase and an endopolymethylgalacturonase. An endopectic acid transeliminase from soil, which catalysed the degradation of pectic acid to predominantly an unsaturated trigalacturonic acid,[43] attacked the glycosidic bond of the terminal nonreducing galacturonosyl residue of tetragalacturonic acid twenty-six times faster than it attacked the middle glycosidic bond.[44] The ratio of the rates with the unsaturated tetramer was only 1·6 : 1. A purified polygalacturonate *trans*-eliminase of *Xanthomonas campestris* catalysed the random cleavage of polygalacturonic acid but was inactive towards fully esterified polymethylpolygalacturonate methyl glycoside.[45] The degradation products from polygalacturonic acid at 46%-breakdown were unsaturated di- and trigalacturonic acids and saturated mono-, di-, and tri-galacturonic acids. Pentagalacturonic acid was degraded preferentially to a saturated dimer and unsaturated trimer, or to saturated trimer and unsaturated dimer. Monomer and unsaturated tetramer were formed more slowly.

A galactoglucomannan extracted with hot water from pinewood contained galactose, glucose, and mannose in the molecular proportions 1 : 1 : 3.[46] Methylation studies indicated a branched structure, similar to that of galactoglucomannans from other resinous woods, in which sequences of (1 → 4)-linked glucosyl and mannosyl residues were substituted at C-6 by short chains containing galactopyranosyl residues.

Partial hydrolysis and methylation studies[47] of an electrophoretically homogeneous 4-*O*-methylglucuronoxylan from white willow (*Salix alba* L.) suggested that it contained 120 β-(1 → 4)-linked D-xylopyranosyl residues every eleventh of which, on average, was substituted at C-2 by either D-glucopyranosyluronic acid or its 4-*O*-methyl ether.

Two acidic glucoxylans isolated[48] from the roots of sugar maple (*Acer saccharum*) were structurally distinct from the polysaccharides found in the

[39] H. Kauss, A. L. Swanson, and W. Z. Hassid, *Biochem. Biophys. Res. Comm.*, 1967, **26**, 234.
[40] H. Kauss and W. Z. Hassid, *J. Biol. Chem.*, 1967, **242**, 1680.
[41] R. W. Stoddart, A. J. Barrett, and D. H. Northcote, *Biochem. J.*, 1967, **102**, 194.
[42] L. 'Rexnová-Benková, *Coll. Czech. Chem. Comm.*, 1967, **32**, 4504.
[43] S. Hasegawa and C. W. Nagel, *J. Food Sci.*, 1966, **31**, 838.
[44] C. W. Nagel and S. Hasegawa, *Arch. Biochem. Biophys.*, 1967, **118**, 590.
[45] S. Nasumo and M. P. Starr, *Biochem. J.*, 1967, **104**, 178.
[46] A. J. Roudier and L. Eberhard, *Bull. Soc. chim. France*, 1967, 1741.
[47] Š. Karácsonyi, M. Kubačková, and J. Hrivňák, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3597.
[48] S. J. Scott and G. W. Hay, *Canad. J. Chem.*, 1967, **45**, 2217.

shoot. One of the glucoxylans (DP 144) had a chain of β-(1 → 4)-linked D-glucosyl and D-xylosyl residues, some of each of which were branched through C-3. The terminal, nonreducing residues were either D-xylose or an, as yet unidentified, sugar acid. The other glucoxylan (DP 96) had a similar structure but was branched through some of the D-xylosyl residues only.

Hydrolysis and methylation studies of hemicellulose of sisal fibre (*Agave sisalana*) indicated[49] a structure of chains of approximately 97 (1 → 4)-linked β-D-xylopyranosyl residues, of which approximately every eighth residue was substituted at C-2 by 4-*O*-methyl-D-glucopyranosyluronic acid. The xylan contained a small number of branch points. Hydrolysis studies suggested[50] that the most probable sequence of sugars in heconin, from the leaves of *Agave sisalana*, is hecogenin–glucose–rhamnose–galactose–xylose–fructose–rhamnose–glucose.

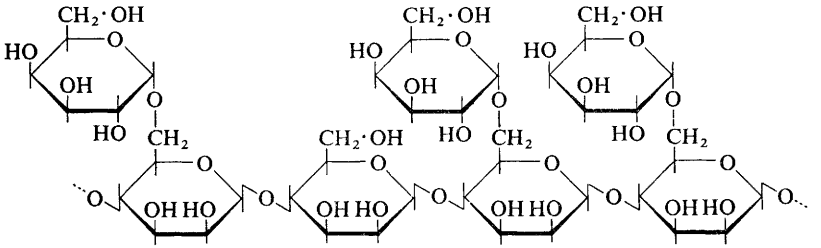
A xylan extracted by alkali from perennial rye-grass (*Lolium perenne*) consisted of chains of (1 → 4)-linked β-D-xylopyranosyl residues to which were attached residues of 4-*O*-methyl-D-glucuronic acid and L-arabinofuranose linked through C-2 and C-3, respectively, of the xylosyl residues.[51] Similar structures have been proposed for the xylans isolated from cocksfoot grass[52] and straw of cereals.[53] Serological cross-reactions between unrelated pollens might be due to the presence of arabinogalactans which are probably components of the cell-wall of pollens.[54]

A galactomannan from seeds of *Anthyllis vulneraria* L. liberated D-mannose (1·32 mol.) and D-galactose (1·0 mol.) on complete hydrolysis and on partial hydrolysis gave 4-*O*-β-D-mannopyranosyl-D-mannose (mannobiose) and 6-*O*-α-D-galactopyranosyl-D-mannose as the only disaccharides, together with tri- and tetra-saccharides of the form (D-mannosyl)$_n$-D-galactose.[55] These results, together with those from periodate oxidation studies and hydrolysis by β-galactosidase, supported the formula (4) for the galactomannan which is in good agreement with the general structure of galactomannans from *Leguminosae*.

Arabic acid and certain polysaccharides from defatted soy-bean flour, defatted jack bean flour, and maize flour formed precipitates with the dye, 1,3,5-tri-[*p*-(β-D-glucosyloxy)phenylazo]-2,4,6-trihydroxybenzene.[56] The polysaccharides could be recovered from solutions of the precipitates in alkaline sodium chloride solution by chromatography on Sephadex G50. A glycoprotein, in which an arabinogalactan and an arabinoxylan were

[49] P. C. Das Gupta and P. P. Mukherjee, *J. Chem. Soc.* (*C*), 1967, 1179.
[50] O. Elsner, *Israel J. Chem.*, 1967, **5**, 60P.
[51] M. Alam and R. J. McIlroy, *J. Chem. Soc.* (*C*), 1967, 1577.
[52] G. O. Aspinall and I. M. Cairncross, *J. Chem. Soc.*, 1960, 3877.
[53] G. O. Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 437; *Ann. Rev. Biochem.*, 1962, **31**, 79.
[54] L. S. Kind and B. Nilsson, *Immunology*, 1967, **13**, 477.
[55] R. Sømme, *Acta Chem. Scand.*, 1967, **21**, 685.
[56] J. Yariv, H. Lis, and E. Katchalski, *Biochem. J.*, 1967, **105**, 1C.

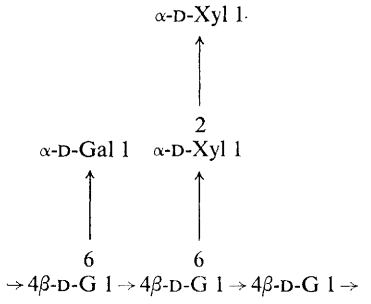
linked by a polypeptide, has been isolated [57a] from wheat flour, and a protein–polysaccharide complex containing hydroxyproline, serine, and threonine has been isolated [57b] from corn pericarp (see also p. 209).



D-galactosyl residues connected to 76% of the D-mannosyl residues of the backbone chain

(4)

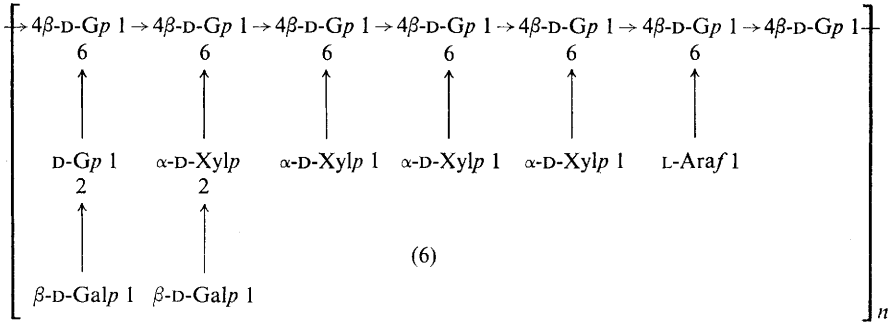
Nasturtium amyloid contained D-glucopyranosyl, D-xylopyranosyl, and D-galactopyranosyl residues (3 : 2 : 1) in a structure [58] (5) similar to that



(5)

proposed [59] for a polysaccharide from the seed kernel of tamarind (*Tamarinus indica*). More recent structural studies [60] of an electrophoretically homogeneous polysaccharide from tamarind kernel by acetolysis, enzymic degradation, methylation, Smith degradation, partial hydrolysis with acid, and methylation of the product suggested (6) as a possible structure for the polysaccharide. No information was available on the sequential distribution of substituents along the main, cellulose-type chain. A mannan isolated from the kernel of daum palm (*Hyphaene thebaica*)

[57a] H. Neukom, L. Providoli, H. Gremli, and P. A. Hui, *Cereal Chem.*, 1967, **44**, 238.
[57b] J. A. Boundy, J. S. Wall, J. E. Turner, J. H. Woychick, and R. J. Dimler, *J. Biol. Chem.*, 1967, **242**, 2411.
[58] D.-S. Hsu and R. E. Reeves, *Carbohydrate Res.*, 1967, **5**, 202.
[59] N. A. Khan and B. P. Mukkerjee, *Chem. and Ind.*, 1959, 1413.
[60] H. C. Srivistava and P. P. Singh, *Carbohydrate Res.*, 1967, **4**, 326.



(7) GalA—GalA—GalA—Rha—GalA—Rha—GalA—Rha—GalA—GalA—Rha—Rha—GalA—

3 4 4 3 4

Interior chain type

(8) β-D-GpA 1→4-D-Galp
(9) β-D-GpA 1→6-D-Galp 1—
(10) β-D-Galp 1→2-D-Xylp

Side chains

(11) β-D-Xylp (linked to C-3 of D-GalA)
(12) β-D-Galp 1→[→4β-D-Galp]$_n$ (at least 8 units)
(13) α-L-Fucp 1→2-D-Xylp 1—
(14) L-Araf 1→5-L-Araf 1—
(15) β-D-GpA 1→4-L-Fucp

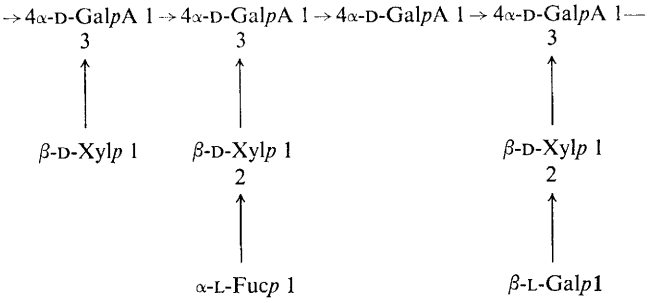
contained D-galactose (5%) and D-mannose (95%), the latter linked β-(1 → 4).[61]

A low molecular weight (DP 11) arabinogalactan, containing galactose (25 parts) and arabinose (2 parts), and a mannan have been isolated [62] from instant coffee powder.

The structures of polysaccharides isolated from the cotyledon meal and hulls of soy-beans have been investigated. The cotyledon meal contained an arabinogalactan and an acidic polysaccharide complex.[63] The former contained chains of (1 → 4)-linked β-D-galactopyranosyl residues in which every fourth or fifth residue was substituted at C-3 by a side-chain carrying, on average, two (1 → 5)-linked L-arabinofuranosyl residues. The acidic polysaccharide complex was highly branched and contained residues of L-fucose, L-rhamnose, D-xylose, L-arabinose, D-galactose, and D-galacturonic acid. Partial hydrolysis with acid liberated the following oligosaccharides: 4-*O*-(β-D-galactopyranosyl)-D-galactose and the polymer-homologous tri-, tetra-, penta-, and hexa-saccharides; 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid and the polymer-homologous trisaccharide; 2-*O*-(β-D-galactopyranosyluronic acid)-L-rhamnose; a tetrasaccharide containing alternating residues of D-galacturonic acid and L-rhamnose, and small amounts of three aldobiouronic acids containing D-glucuronic acid.[64] Among the oligosaccharides isolated by partial acetolysis of the acetylated polysaccharide complex, followed by deacetylation, were 2-*O*-(α-L-fucopyranosyl)-D-xylose, 2-*O*-(β-D-galactopyranosyl)-D-xylose, and acidic oligosaccharides containing D-glucuronic acid and contiguous L-rhamnosyl residues. Enzymic degradation of a degraded polysaccharide produced by acid hydrolysis (0·05N-sulphuric acid at 100° for 5·5 hr.) liberated 3-*O*-(β-D-xylopyranosyl)-D-glucuronic acid and higher acidic oligosaccharides containing chains of (1 → 4)-linked β-D-galactopyranosyl residues. These studies suggested [64] that the acidic polysaccharide complex has an interior chain of the type (7). Partial structures of oligosaccharide side-chains are shown in (8) to (15). Several of these structural features have been found in some pectinic acids and tragacanthic acid. Partial hydrolysis with acid of an arabinogalactan extracted from soy-seeds with hot water gave a homologous series of galacto-oligosaccharides, two of which have been characterised [65] as (1 → 4)-linked di- and tri-galactoses. The physical constants of the disaccharide were in agreement with those of 4-*O*-(β-D-galactopyranosyl)-D-galactose. These conclusions support the findings of Aspinall *et al.*[64]

[61] H. El Khadem and M. A. E. Sallam, *Carbohydrate Res.*, 1967, **4**, 387.
[62] M. L. Wolfrom and L. E. Anderson, *J. Agric. Food Chem.*, 1967, **15**, 685.
[63] G. O. Aspinall, R. G. Begbie, A. Hamilton, and J. N. C. Whyte, *J. Chem. Soc.* (*C*), 1967, 1065.
[64] G. O. Aspinall, I. W. Cottrell, S. V. Egan, I. M. Morrison, and J. N. C. Whyte, *J. Chem. Soc.* (*C*), 1967, 1071.
[65] M. Morita, M. Okuhara, T. Kikuchi, and Y. Sakurai, *Agric. and Biol. Chem.* (*Japan*), 1967, **3**, 314.

Partial hydrolysis or partial acetolysis of acidic polysaccharides isolated from the hulls of soy-bean revealed [66] several structural features in common with the acidic polysaccharide complex isolated from soy-bean cotyledon meal. The structurally related acidic polysaccharides in the hulls comprised a group of pectic acid-type of polysaccharides in which interior chains of residues of 4-*O*-substituted D-galacturonic acid and 2-*O*-substituted L-rhamnopyranose had side-chains composed mainly of neutral sugar residues. The latter included L-arabinosyl residues, 2-*O*-(α-L-fucopyranosyl)-D-xylopyranose, 2-*O*-(β-D-galactopyranosyl)-D-xylopyranose, and chains of (1 → 4)-linked β-D-galactopyranosyl residues. Partial hydrolysis of two of the acidic polysaccharides liberated 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, a structural sequence which hitherto had only been detected among polysaccharides of plant gums.



(16)

The structure (16) previously proposed [67] for gum tragacanth was modified slightly by the characterisation [68] of the aldobiouronic acids 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(β-D-glucopyranosyluronic acid)-L-fucose, and 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose as minor products of partial hydrolysis with acid of tragacanthic acid. Small proportions of the sequences α-D-glucopyranosyluronic acid (1 → 4)-β-D-galactopyranosyl (1 → 2)-β-D-xylopyranose and β-D-glucopyranosyluronic acid (1 → 4)-α-L-fucopyranosyl (1 → 2)-β-D-xylopyranose were also present in tragacanthic acid. Thus the core structure was a galacturonorhamnan with a very small proportion of L-rhamnosyl residues.

The gum of *Virgilia oroboides*, which contains D-glucuronic acid and its 4-*O*-methyl ether, liberated 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose on partial hydrolysis.[69] A third aldobiouronic acid has been isolated and characterised as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. *i.e.* the

[66] G. O. Aspinall, K. Hunt, and I. M. Morrison, *J. Chem. Soc.* (*C*), 1967, 1080.
[67] G. O. Aspinall and J. Baillie, *J. Chem. Soc.*, 1963, 1702.
[68] G. O. Aspinall, D. B. Davies, and R. N. Fraser, *J. Chem. Soc.* (*C*), 1967, 1086.
[69] A. M. Stephen, *Carbohydrate Res.*, 1967, **5**, 335.

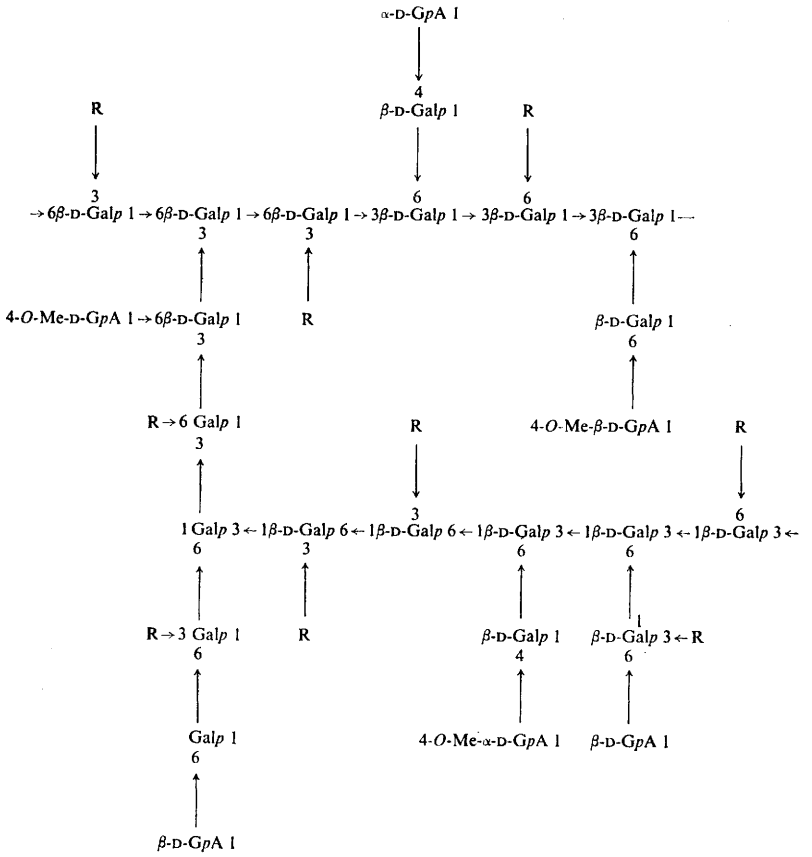
analogue of the major aldobiouronic acid in which the D-galactosyluronic acid moiety is *O*-methylated at C-4. The same three aldobiouronic acids, together with the dimer, trimer, and tetramer of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, have been isolated [70] by graded hydrolysis of the gum exudate of *Encephalartos longifolius*. Methylation and methanolysis studies of this gum showed that it contained structurally complex, highly branched polysaccharides in which unsubstituted chain units comprised less than one-third of the sugar residues. Residues of L-rhamnopyranose, its 3-*O*-methyl ether, L-arabinofuranose, and some residues of D-mannopyranose were present as end-groups. L-Arabinopyranosyl and probably D-xylopyranosyl residues were also located in the periphery of the molecule. The carbohydrate chains comprised D-galactopyranosyl units, linked through C-3 and C-6, and some of the D-mannopyranosyl units, linked through C-2 and C-3. Residues of uronic acid were present either as non-reducing end-groups or as chain units substituted at C-4 by D-mannose and linked β-(1 → 6) and β-(1 → 2) to D-galactosyl and D-mannosyl residues, respectively. Mild hydrolysis with acid liberated 3-*O*-methyl-L-rhamnose (3%), L-rhamnose (14%), L-fucose (0·4%), D-xylose (4%), L-arabinose (8%), D-mannose (0·5%), and D-galactose (8%). The residual material contained D-glucosyluronic acid residues linked β-(1 → 6) to D-galactose, and mannose-containing acidic oligosaccharides.

Further structural information on sapote gum, which contained D-xylose, L-arabinose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid in the molecular proportions 2·2 : 1·0 : 0·42 : 0·58, was obtained [71] by characterisation of two crystalline aldotriouronic acids isolated after partial hydrolysis with acid. One was identified as *O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose by comparison with the authentic trisaccharide, and the other as *O*-α-D-glucopyranosyluronic acid-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose by partial hydrolysis, reduction, and methylation studies.

Further structural studies of several *Acacia* gum polysaccharides have been reported. Certain *Acacia* gum polysaccharides containing significant proportions of L-rhamnopyranosyl end-groups linked to C-4 of D-glucuronic acid were degraded during methylation with the sodium hydride–methyl iodide–DMSO system.[72] Earlier indications that the gum of *Acacia karroo* Hayne contained residues of D-glucuronic acid linked β-(1 → 6) and α-(1 → 4) to D-galactose have been substantiated.[73] Most of the residues of hexuronic acid were present as end groups. Similar structural features were present in *A. arabica* gum.[74] Methylation, and hydrolysis and Smith degradation

[70] A. M. Stephen and D. C. de Bruyn, *Carbohydrate Res.*, 1967, **5**, 256.
[71] R. D. Lambert, E. E. Dickey, and N. S. Thompson, *Carbohydrate Res.*, 1968, **6**, 43.
[72] D. M. W. Anderson, I. C. M. Dea, P. A. Maggs, and A. C. Munro, *Carbohydrate Res.*, 1967, **5**, 489.
[73] A. M. Stephen and D. C. Vogt, *Tetrahedron*, 1967, **23**, 1473.
[74] D. M. W. Anderson, Sir Edmund Hirst, and J. F. Stoddart, *J. Chem. Soc.* (*C*), 1967, 1476.

studies of the latter gum, and of a degraded gum produced by controlled hydrolysis with acid, suggested that the galactan framework (17) in



R represents L-Ara-containing side-chains. Some are at least 6 units long and contain (1→3)-linked L-Ara*f*; (1→2)-linked L-Ara*f* and (1→2)-linked L-Ara*p* residues. May be terminated by L-Ara*f* or L-Ara*p* residues.

A. arabica was more highly branched, and therefore more compact, than that of *A. senegal* gum and that the L-arabinose-containing side-chains were longer than in *A. senegal* gum. Physicochemical measurements on solutions of the two gums supported these conclusions.

Light-scattering studies [75] on the sodium salt of whole gum from authentic *A. senegal* gave $\overline{M}_W = 580{,}000$. $\overline{M}_W$ Values for three molecular-weight fractions obtained by precipitation with sodium sulphate confirmed that

[75] D. M. W. Anderson, Sir Edmund Hirst, S. Rahman, and G. Stainsby, *Carbohydrate Res.*, 1967, **3**, 308.

the gum had a broad molecular-weight distribution. It was pointed out [76] that values for modified Staudinger constants, calculated from the relationship between viscosity and molecular weight for *A. senegal* gum, would not be generally applicable to other *Acacia* species. Subsequent analytical studies [77] of different forms of the gum from *A. senegal* Willd emphasised that commercial gum samples should not be used for fundamental studies. Single nodule specimens showed considerable analytical variations. A sample of 'Hennawi' gum, from the main trunk of the *A. senegal* tree, contained less L-rhamnosyl and fewer free uronic carboxy-groups than normal.[78]

Water-soluble, salt-soluble, and alkali-soluble polysaccharides isolated from the gum polysaccharide of *A. drepanolobium* each contained [79] four aldobiouronic acids: 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose; 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose, 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

Hydrolysis, periodate oxidation, and enzymic degradation studies of phytoglycogen from wild apples (*Malus silvestris* Mill.) indicated [80] a highly branched α-glucan structure with α-(1 → 4) and α-(1 → 6)-D-glucosidic bonds. The average length of external chains in the molecule was 4·25 D-glucosyl units and that of internal chains was 2·0 D-glucosyl units.

Amylose polyalcohols, produced by prolonged oxidation of potato and corn amylose with periodate followed by borohydride reduction, liberated 1,4-di-*O*-methyl-D-erythritol and a small proportion of 1-*O*-methyl-D-erythritol after sequential methylation and methanolysis.[81] These results supported the view that amylose from both sources contained (1 → 4, 6 ← 1)-D-glucosyl branch points. Two preparations of 6-*O*-methyl-amylose, in which the degree of substitution of primary alcohol groups was 0·2 and 0·4, were resistant to degradation by sweet-potato β-amylase and potato phosphorylase, but were degraded by an α-amylase preparation.[82] Hydrolysis was blocked in the vicinity of the methylated primary hydroxy-groups and it was concluded that the limitations on amylase action, normally attributed to (1 → 4, 6 ← 1)-D-glucosyl branch points, are also imposed by the smaller methyl group.

A minor component of the water-soluble, nonstarchy polysaccharides of the endosperm of naked barley has been identified,[83] by methylation studies, as a glucan with a main chain of (1 → 4)- and (1 → 3)-linked

[76] D. M. W. Anderson and S. Rahman, *Carbohydrate Res.*, 1967, **4**, 298.
[77] D. M. W. Anderson, I. C. M. Dea, K. A. Karamalla, and J. F. Smith, *Carbohydrate Res.*, 1968, **6**, 97.
[78] D. M. W. Anderson, and I. C. M. Dea, *Carbohydrate Res.*, 1968, **6**, 104.
[79] D. M. W. Anderson and I. C. M. Dea, *Carbohydrate Res.*, 1967, **5**, 461.
[80] K. Babor, V. Kaláč, K. Tihlárik, and J. Rosik, *Coll. Czech. Chem. Comm.*, 1967, **32**, 3071.
[81] A. Misaki and F. Smith, *Carbohydrate Res.*, 1967, **4**, 109.
[82] C. E. Weill and M. Brutt, *Carbohydrate Res.*, 1967, **4**, 230.
[83] O. Igarashi, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 578.

β-D-glucopyranosyl residues in the ratio 2 : 1 and some branching. A mixture of glucofructans, extracted from the endosperm with 70% aqueous ethanol, was fractionated by chromatography of the acetates.[84] Analytical and structural information on the glucofructans is summarised in (18).

Glucofructan	D-fructose/ D-glucose	Structure
1	1·8	D-G*p* 1→2-D-Fru*f* 1→2-D-Fru*f*
2	3·8	either D-G*p* 1→2-D-Fru*f* 1→2-D-Fru*f* 1→2-D-Fru*f* 6 ↑ D-Fru*f* 1
		or D-G*p* 1→2-D-Fru*f* 1→2Fru*f* 1→2Fru*f* 6 ↑ D-Fru*f* 1
3	5·0 (DP = 6)	(18)
4	6·1 (DP = 7)	

The mucilage of the yam (*Dioscorea batas decne forma* Icho) comprised a phosphomannan protein complex, $[\alpha]_D^{24} - 60°$, M_W $14{\cdot}6 \times 10^4$, mannan 48%, P 3·8%, and protein 10%.[84] The mannan was β-(1→4)-linked and contained 2 mol. of acetyl per mol. of anhydromannose.[85] The phosphorus content was attributed to the presence of phytic acid and the protein component was rich in hydroxyamino-acid residues.[86]

[84] T. Shin and Y. Sakurai, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 879.
[85] T. Satoh, *J. Chem. Soc. Japan*, 1967, **88**, 982, 989.
[86] T. Satoh, *J. Chem. Soc. Japan*, 1967, **88**, 985.

4
Bacterial Polysaccharides

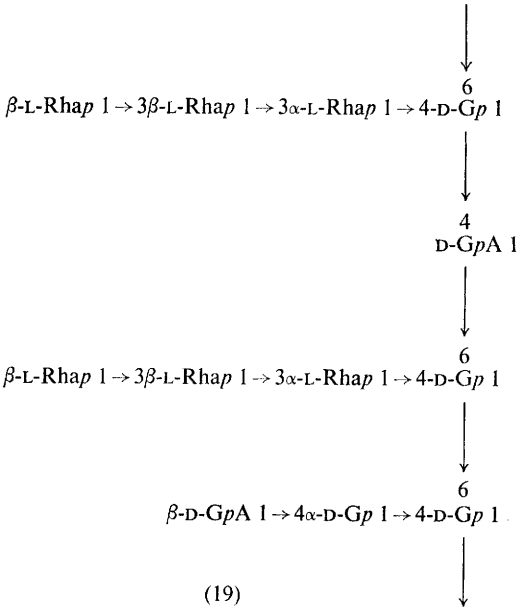
The capsular polysaccharide of *Pneumococcus* Type I (SI) has been purified by chromatography on DEAE-Sephadex and by recovery from its specific precipitate with homologous antibody.[87] Electrophoretically and immunochemically homogeneous polysaccharide SI, $\{[\alpha]_D +266°$; anhydrohexuronic acid, 67%; N, 5%; total acetyl, 8·8%; *O*-acetyl, 3·1%}, resisted oxidation by periodate. Deamination with nitrous acid gave at least six products, two of which indicated that SI contained residues of the disaccharides 2-amino-2-deoxy-(D-galactosyluronic acid)-galactose and 2-amino-2-deoxy-(D-galactosyluronic acid)-glucose. 2-Amino-2-deoxyhexosyl-D-glucose residues were also indicated as components of the polysaccharide and an oligosaccharide from partially carboxy-reduced SI was tentatively assigned the partial structure D-galactosyluronic acid-(1 → 3)-2-amino-2-deoxy-glucosyl-(1 → 3)-D-galactosyluronic acid. The presence of D-glucose as a component monosaccharide of SI was confirmed.

Further information about the structure of the capsular polysaccharide of Type II *Pneumococcus* (SII) has been obtained[88] by degradation of SII and its carboxy-reduced derivative with specific, induced α- and β-D-glucosidases and α-L-rhamnosidase. Those results coupled with data from periodate-oxidation studies suggested structure (19) as a possible repeating unit for SII.

The antigenic cross-reactivity between the carbohydrates of haemolytic streptococci groups B and G is due to the occurrence of multiple residues of L-rhamnose in both polysaccharides.[89] Since these same polysaccharides cross-reacted with Type XXIII antipneumococcal serum, L-rhamnose, probably in the form of terminal, nonreducing residues, was implicated as a determinant sugar of the *Pneumococcus* Type XXIII polysaccharide (SXXIII).[90] This was endorsed by the observations that partial hydrolysis of SXXIII with 0·01 N-sulphuric acid, which resulted in removal of much L-rhamnose, was accompanied by a considerable loss in serological activity, and that L-rhamnose inhibited the cross-reaction between streptococcal

[87] R. C. E. Guy, M. J. How, M. Stacey, and M. Heidelberger, *J. Biol. Chem.*, 1967, **242**, 5106.
[88] S. A. Barker, P. J. Somers, and M. Stacey, *Carbohydrate Res.*, 1967, **3**, 261.
[89] S. N. Curtis and R. M. Krause, *J. Exptl. Med.*, 1964, **120**, 629.
[90] M. Heidelberger, J. M. Davie, and R. M. Krause, *J. Immunol.*, 1967, **99**, 794.

group B antigen and anti-Pn XXIII and the SXXIII anti-SXXIII reaction. D-Galactose was also involved in the specificity of SXXIII and preliminary chemical analysis [91] of the polysaccharide indicated the presence of rhamnose, galactose, and glucose (5 : 2 : 2).



(19)

Studies of the precipitation of pneumococcal Type II antiserum (anti-Pn II) by *E. coli* M-11 polysaccharide and a dextran demonstrated the heterogeneity of the antibodies in the serum.[92] The cross-precipitation by the *E. coli* polysaccharide was probably due to the presence of multiple residues of nonreducing end groups of D-glucuronic acid. Dextran, however, precipitated a different fraction of the Type II antibodies and keyhole limpet haemocyanin-*ortho*-azophenyl-β-D-glucuronide precipitated all the Pn II antibodies. Each cross-reacting antibody fraction had the capacity to react with D-glucosyluronic acid residues, but each fraction had an additional specificity with was met by either dextran or the *E. coli* polysaccharide, but not by both.

Structural studies of pneumococcal C-substance, a constituent common to all pneumococci, have been continued by two schools.[93, 94] Gotschlich

[91] J. K. N. Jones and M. B. Perry, *unpublished results cited in ref.* 90.
[92] S. Zolla and J. W. Goodman, *Immunochemistry*, 1967, **4**, 135.
[93] E. C. Gotschlich and T.-Y. Liu, *J. Biol. Chem.*, 1967, **242**, 463.
[94] D. E. Brundish and J. Baddiley, *Biochem. J.*, 1967, **105**, 30c.

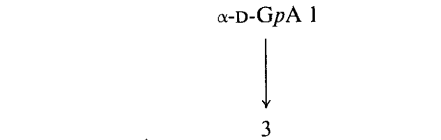
and Liu[93] concluded that the major serological determinant of the C-substance was a polymer with a repeating unit of 2-acetamido-2-deoxy-β-D-galactose 1-phosphate residues linked through its phosphate to the C-6-hydroxy-group of the neighbouring sugar residue. It was proposed that the polymer, which reacts with human C-reactive protein, was cross-linked to a mucopeptide which contained 2-amino-3-*O*-(2-carboxyethyl)-2-deoxy-D-glucose 6-phosphate (muramic acid 6-phosphate) and 2-acetamido-2-deoxy-D-glucose. 2-Amino-3-*O*-(2-carboxyethyl)-2-deoxy-D-glucose phosphate has also been reported[95] in hydrolysates of cell-walls obtained from all of eight other gram-positive bacteria investigated. Brundish and Baddiley[94] reported a more complex structure for pneumococcal C-substance which they classified as a ribitol techoic acid. Their preparations contained, in addition to 2-acetamido-2-deoxy-D-galactose 6-phosphate, residues of ribitol phosphate and an acid-labile 2-acetamido-4-amino-2,4,6-trideoxyhexose, previously reported[96] to be incorporated into C-polysaccharide from its uridine nucleotide. Small amounts of D-glucose and choline were also components of the polymer. C-Polysaccharide of the same composition [P: 2-amino-2-deoxy-D-galactose : D-glucose : N (1·00 : 0·79 : 0·18 : 2·85)] was isolated from several different rough and capsulated pneumococcal types. Structural studies using acid and alkaline hydrolysis, treatment with phosphomonoesterase, and periodate oxidation suggested a repeating unit of (2-acetamido-2-deoxy-D-galactosyl)-acetamido-diamino-trideoxy-hexosyl 5-phosphate, joined through its phosphate group to either C-3 or C-4 of the 2-amino-2-deoxy-D-galactosyl residue of an adjacent repeating unit. The repeating unit also contained 0·2 molecular proportions of D-glucose and some choline. The D-glucosyl residues were destroyed by periodate oxidation.

Phage-infected cells of encapsulated *Klebsiella aerogenes* Types 54 [A3(S1)] produced fucosidases which catalysed the release from the capsular polysaccharide of approximately equal amounts of two tetrasaccharides,[97] neither of which was identical with oligosaccharides released on autohydrolysis. Both tetrasaccharides contained D-glucose, glucuronic acid, and fucose (2 : 1 : 1), with fucose as the terminal, reducing sugar. Structural studies suggested[97] that both contained cellobiose and a glucosyluronic acid-fucose disaccharide, and that one tetrasaccharide was an *O*-acetyl derivative of the other.

Structural studies showed[98] that the four serologically identical capsular polysaccharides produced by the NCTC 243, 418, 5055, and 9504 strains of *Aerobacter aerogenes* were identical and contained a tetrasaccharide repeating unit (20). Detailed quantitative examination of the 243 polysaccharide involved methylation, partial hydrolysis, periodate oxidation,

[95] T.-Y. Liu and E. C. Gotschlich, *J. Biol. Chem.*, 1967, **242**, 471.
[96] J. Distler, B. Kaufman, and S. Roseman, *Arch. Biochem. Biophys.*, 1966, **116**, 466.
[97] I. W. Sunderland, *Biochem. J.*, 1967, **104**, 278.
[98] L. C. Gahan, P. A. Sandford, and H. E. Conrad, *Biochemistry*, 1967, **6**, 2755.

and ^{1}H n.m.r. studies. A ^{14}C-labelled polysaccharide was isolated from cultures grown on ^{14}C-D-glucose as the sole carbon source. Partial hydrolysis of the ^{14}C-labelled polysaccharide, followed by reduction of the oligosaccharides produced with borotriti-ide permitted calculation of the degree



(20)

of polymerisation of the oligosaccharides by measurement of their contents of ^{14}C and ^{3}H. A fully methylated neutral polysaccharide produced by reduction with lithium aluminium triti-ide was used to calculate the percentage of uronic acid, since each hexosyluronic acid residue incorporated two non-exchangeable tritium atoms on reduction to the neutral sugar. The structure proposed for the *A. aerogenes* polysaccharides differed from one postulated by Barker and Siddiqui [99] who found D-mannuronic acid as a component monosaccharide of a so-called 418 strain that had several different properties (*e.g.* $[\alpha]_D$ and ratio D-glucose : D-mannose) from the NCTC strain.

The capsular polysaccharide of *Klebsiella pneumoniae* Type 2(B) contained D-glucose, mostly (1 → 3)-linked but some as branch points in the 4 or 6 positions, (1 → 6)-linked D-glucuronic acid, and a small amount of D-mannose which could be the nonreducing residue at the branch point.[100]

A homogeneous Type I antigen purified from the autolytic digest of cell-walls of group D streptococci contained glucose, rhamnose, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose together with phosphorus and residual peptidoglycan components.[101] It was proposed that the antigen was a heteropolymer which might contain a ribitol teichoic acid. The capsular type-specific polysaccharide extracted from group B Type II streptococcus with cold trichloroacetic acid contained D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and an, as yet, unidentified labile component.[102] A partial antigen containing terminal, nonreducing D-galactosyl residues could be extracted with dilute hydrochloric acid at 100°. Both the group-specific and the type-specific carbohydrate antigen have been isolated from a formamide extract of a Type II strain of group F streptococcus.[103] The component sugars of the Type II polysaccharide were galactose, glucose, 2-amino-2-deoxygalactose and rhamnose whereas the

[99] S. A. Barker and I. R. Siddiqui, *J. Chem. Soc.*, 1958, 2358.
[100] S. H. Park, J. Eriksen, and S. D. Henriksen, *Acta Path. Microbiol. Scand.*, 1967, **69**, 431.
[101] A. S. Bleiweis, F. E. Young, and R. M. Krause, *J. Bacteriol.*, 1967, **94**, 1381.
[102] E. H. Freimer, *J. Exptl. Med.*, 1967, **125**, 381.
[103] M. F. Michel and R. M. Krause, *J. Exptl. Med.*, 1967, **125**, 1075.

group F antigen contained rhamnose, glucose, 2-amino-2-deoxygalactose, and a small percentage of 2-amino-2-deoxyglucose. The Type II antigen and a group-like antigen have also been isolated [103] from a strain of streptococcus which lacked a serologically detectable streptococcal group antigen. The group-like antigen contained rhamnose, galactose, and 2-amino-2-deoxyglucose and did not cross-react with group F serum. A disaccharide, tentatively identified as 2-acetamido-2-deoxy-3-*O*-(2-acetamido-2-deoxy-α-D-glucosyl)-D-galactose, has been isolated [104] from partial acid hydrolysates of formamide extracts of Z3 streptococcus with and without the Type III antigen. Serological studies showed that the disaccharide was an important part of the Z3 antigen. The Type III antigen contained glucose, galactose, and rhamnose (5 : 3 : 1) whereas the Z3 antigen contained rhamnose, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose (2 : 1 : 1).

The pronounced immunological cross-reaction observed between the group A streptoccal polysaccharide and structural glycoproteins isolated from heart valves suggested [105] that group A streptococcal infection might lead to the production of antivalvular autoantibodies which were observed in the sera of patients with rheumatic fever. Related studies [106] showed that rabbits immunised with mucopeptide from *Streptococcus agalactiae* developed antibodies which cross-reacted with extracts of bovine heart, skeletal muscle, lymph node, and blood.

The K antigen from *E. coli* O9:K30:H12 contained equimolar proportions of glucuronic acid, galactose, and mannose together with *O*-acetyl groups (2·5%).[107] The results of acid and alkaline hydrolyses and periodate oxidation studies indicated that the polysaccharide comprised linear subunits, *M* 150,000, composed of the trisaccharide repeating unit *O*-mannosyl (1 → 2)-β-glucosyluronic acid (1 → 3) galactose, which were joined through ester linkages between carboxy-groups of glucosyluronic acid residues and hydroxy-groups of sugar moieties. The capsular antigen from *E. coli* O9:K9:H12 contained galactose and 2-amino-2-deoxyhexose in the molar ratio 2 : 1, in addition to a component, tentatively identified as sialic acid, which was not cleaved, however, by neuraminidase.[108] The structure of the branched, slime polysaccharide of *E. coli* K12, containing glucuronic acid, fucose, glucose, galactose, and a hitherto unknown, and acid-labile, sugar (ALS) located at chain termini, has been studied [16] by alkaline degradation. The sequence at the end of most, and possibly all, chains was established as ALS-D-glucosyluronic acid-D-galactose. ALS might be a dicarbonyl sugar.

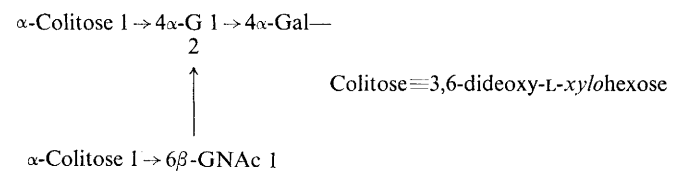
[104] J. M. N. Willers and G. H. J. Alderkamp, *J. Gen. Microbiol.*, 1967, **49**, 41.
[105] I. Goldstein, B. Halpern, and L. Robert, *Nature*, 1967, **213**, 44.
[106] K. Dodd and N. L. Norcross, *J. Bacteriol.*, 1967, **93**, 577.
[107] D. Hungerer, K. Jann, B. Jann, F. Ørskov, and I. Ørskov, *European J. Biochem.*, 1967, **2**, 115.
[108] A. P. MacLennan, E. C. A. Seneviratne, and D. C. Hawkins, *Biochem. J.*, 1967, **102**, 8P.

Structural studies of the Vi-antigen of *Citrobacter freundii* (*E. coli*) 5396/38 using methylation followed by reduction and reduction prior to methylation conclusively demonstrated [109] the presence of (1 → 4)-linked residues of 2-amino-2-deoxy-D-galacturonic acid in the polysaccharide.

4-Amino-4,6-dideoxy-D-glucose and 4-amino-4,6-dideoxy-D-galactose have been identified [110] as component monosaccharides of lipopolysaccharides from two strains of *E. coli*. This was the first report of the latter sugar as a polysaccharide constituent. A component of *E. coli* 071 has been tentatively identified [111] as a 3-amino-3,6-dideoxyglucose; this sugar has also been found in lipopolysaccharides of *Citrobacter* [112] and *Salmonella*;[113] 3-amino-3-deoxy-D-glucose was isolated [114] in yields of 2·6 g./l. from a fermentation broth of a *Bacillus* designated as *B. aminoglucosidicus*. Mild hydrolysis with acid of a lipopolysaccharide from *E. coli* K-12 released a component tentatively identified as a 5-*O*-rhamnosyl 2-oxo-3-deoxyoctonate.[115] The same compound was present in *E. coli* JE1538 but not in a mutant strain defective in UDP-galactose.

The 2-oxo-3-deoxyoctonate component of the lipopolysaccharide from *E. coli* (2101-R) has been characterised [116] as 3-deoxy-D-*manno*-oct-2-ulosnic acid by oxidation with ceric sulphate followed by borohydride reduction of the product to 2-deoxy-D-*manno*-heptose.

Acid hydrolysis and Smith degradation studies of the *O*-antigenic polysaccharide of *E. coli* 0111-B_4 indicated [117] structure (21) for the antigenically active oligosaccharide subunit of the lipopolysaccharide. In the



GNAc residues also substituted at C-3 or C-4 in intact polymer

(21)

intact polymer 2-acetamido-2-deoxyglucose was substituted at C-3 or C-4. *E. coli* J-5, a mutant of *E. coli* 0111-B_4 lacking UDP galactose-4-epimerase, produced a cell-wall lipopolysaccharide which lacked galactose and colitose

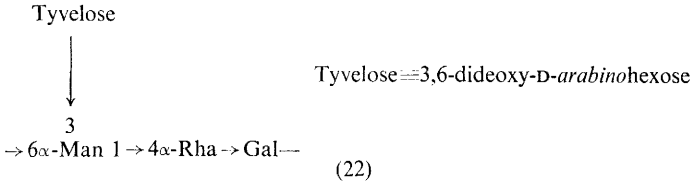
[109] K. Heyns and G. Kiessling, *Carbohydrate Res.*, 1967, **3**, 340.
[110] B. Jann and K. Jann, *European J. Biochem.*, 1967, **2**, 26.
[111] B. Jann, K. Jann, and E. Müller-Seitz, *Nature*, 1967, **215**, 170.
[112] R. A. Raff and R. W. Wheat, *J. Biol. Chem.*, 1967, **242**, 4610.
[113] O. Lüderitz, E. Ruschmann, O. Westphal, R. Raff, and R. Wheat, *J. Bacteriol.*, 1967, **93**, 1681.
[114] S. Umezawa, K. Umino, S. Shibahara, and S. Omoto, *Bull. Chem. Soc. Japan*, 1967, **40**, 2419.
[115] K. Sugimoto and R. Okazaki, *J. Biochem.*, 1967, **62**, 373.
[116] M. B. Perry and G. A. Adams, *Biochem. Biophys. Res. Comm.*, 1967, **26**, 417.
[117] R. D. Edstrom and E. C. Heath, *J. Biol. Chem.*, 1967, **242**, 4125.

(3,6-dideoxy-L-*xylo*-hexose) and contained reduced quantities of glucose and 2-acetamido-2-deoxyglucose.[118] An enzyme preparation from the cell envelope mutant catalysed the apparent sequential transfer of galactose, glucose, 2-acetamido-2-deoxyglucose, and colitose from their respective nucleotide derivatives to the incomplete polymer.

Growth of the lysine-requiring mutant of *E. coli* 12408 under lysine-limiting conditions resulted in the excretion of a lipopolysaccharide–phospholipid–protein complex in which the lipopolysaccharide was similar in composition to the intracellular lipopolysaccharide isolated from rough strains of *E. coli*, and the phospholipid was mainly phosphatidylethanolamine.[119]

Improved methods have been reported[120] for the production and isolation from *E. coli* O16 of colominic acid, a polymer of *N*-acetyl-D-neuraminic acid, using a simple synthetic medium containing succinate, glycine, ammonium sulphate, and other salts. The colominic acid produced was in the ionized rather than the lactone form and was precipitated from the medium by benzylcetyldimethyl ammonium chloride.

Immunochemical studies indicated[121] that *N*-acetyl-D-neuraminic acid was an immunodominant sugar in *Salmonella ngozi*. From the results of phagic and immunological studies Le Minor and Staub[122] predicted the structure of the repeating unit (22) of the group D_2 polysaccharide of



(22)

Salmonella strasbourg. These conclusions were confirmed[123] by the isolation of the oligosaccharides 4-*O*-(α-D-mannosyl)-L-rhamnose, 6-*O*-(α-D-galactosyl)-D-mannose, and *O*-α-D-galactosyl-(1 → 6)-*O*-α-D-mannosyl-(1 → 4)-L-rhamnose from the polysaccharide. Periodate oxidation studies showed that tyvelose (3,6-dideoxy-D-*arabino*-hexose) was linked to C-3 of D-mannose in the repeating unit which contained no *O*-acetyl functions. The lipid involved in *O*-antigen biosynthesis in *Salmonella* has been identified[124] as a polyisoprenoid compound containing eleven isoprene units that is linked to the *O*-antigen repeating sequence through a pyrophosphate residue.

[118] R. D. Edstrom and E. C. Heath, *J. Biol. Chem.*, 1967, **242**, 3581.
[119] K. W. Knox, J. Cullen, and E. Work, *Biochem. J.*, 1967, **103**, 192.
[120] S. A. Barker, R. G. Jones, and P. J. Somers, *Carbohydrate Res.*, 1967, **3**, 369.
[121] B. Kedzierska, *Bull. Acad. polon. Sci., Ser. Sci. biol.*, 1967, **15**, 385.
[122] L. Le Minor and A. M. Staub, *Ann. Inst. Pasteur*, 1966, **110**, 834.
[123] H. O. Nghiem, G. Bagdian, and A. M. Staub, *European J. Biochem.*, 1967, **2**, 392.
[124] A. Wright, M. Dankert, P. Fennessey, and P. W. Robbins, *Proc. Nat. Acad. Sci., U.S.A.*, 1967, **57**, 1798.

The polysaccharides of various mutants of *Salmonella* and *Shigella* have been investigated further. The smooth (*S*) to rough (R) mutation was shown [125] to have the same biochemical basis in both bacteria in that it resulted from enzyme defects which blocked the incorporation of the *O*-specific side-chains that characterised the smooth lipopolysaccharide. Thus, the rough mutants possessed only the underlying basal structure, which had the same sugar components in both *Salmonella* and *Shigella*. The overall structures, however, were different, as reflected by the lack of serological cross-reactions between the two basal structures. Side-chains isolated from the basal structure of *Shigella flexneri* have been characterised [125] as a trisaccharide sequence 2-acetamido-2-deoxy-α-glucosyl-(1 → 4)-galactosyl-(1 → 3)-glucose in which the 3- and 4-positions of the galactosyl and glucosyl residues, respectively, were substituted by glucose. The analogous oligosaccharide from *Salmonella* had terminal α-galactosyl and 2-acetamido-2-deoxy-α-glucosyl-glucosyl residues. The heptose core of lipopolysaccharides derived from three classes of *Salmonella minnesota* R mutants has been studied by methylation, Smith degradation, and enzymic degradation and the following structures were proposed [126] in the three classes:

class Rd_2 α-heptose—KDO—X
class Rd_1 α-heptose (1 → 3) α-heptose—KDO—X
class Rc glucose (1 → 3) α-heptose (1 → 3) α-heptose—KDO—X

where X contained a 2-oxo-3-deoxyoctonate, ethanolamine, phosphate, and lipid A, and heptose was L-*glycero*-D-*manno*-heptose. L-*Glycero*-D-*manno*-heptose inhibited precipitation in both the Rd_1 and Rd_2 systems [127] as would be expected from the presence of terminal, non-reducing heptosyl units in both Rd_1 and Rd_2 lipopolysaccharides.

Lipopolysaccharides have been isolated from *Salmonella* T mutants, which are intermediate between the *S* and R forms.[128] T1 Lipopolysaccharides contained 14–22% of both D-galactose and D-ribose, in addition to α-2-oxo-3-deoxyoctonate, L-*glycero*-D-*manno*-heptose, D-glucose, and 2-amino-2-deoxy-D-glucose. The D-galactose was probably (1 → 4)-linked and the heptosyl residue either (1 → 3)-linked or branched. The D-ribosyl residues resisted oxidation with periodate. Studies of *Salmonella* mutants deficient in *O*- and R-polysaccharides and heptose suggested that none of those components was functional in the primary toxicity of endotoxin, rather that the toxic moiety was associated with the lipid portion of the molecule.[129]

[125] J. H. Johnston, R. J. Johnston, and D. A. R. Simmons, *Biochem. J.*, 1967, **105**, 79.
[126] W. Dröge, O. Lüderitz, and O. Westphal, *Z. physiol. Chem.*, 1967, **348**, 603.
[127] H. J. Risse, W. Dröge, E. Ruschmann, O. Lüderitz, O. Westphal, and J. Schlosshardt, *European J. Biochem.*, 1967, **1**, 216.
[128] R. W. Wheat, M. Berst, E. Ruschmann, O. Lüderitz, and O. Westphal, *J. Bacteriol.*, 1967, **94**, 1366.
[129] Y. B. Kim and D. W. Watson, *J. Bacteriol.*, 1967, **94**, 1320.

The lipopolysaccharide from *Azotobacter vinelandii* dissociated into smaller homogeneous units on treatment with ethylenediaminetetra-acetate or dodecyl sulphate.[130] A reassociated polymer was obtained by dialysis in the presence of calcium chloride.

Decapsulated cells of *Serratia marcerans* have been fractionated into crude cytoplasmic polysaccharides, lipopolysaccharide, and cell-wall polysaccharides.[131] The cytoplasmic fraction contained dialysable polymers of D-glucose and D-mannose and nondialysable polysaccharides containing D-glucose, D-mannose, L-rhamnose, glucuronic acid, and 2-amino-2-deoxyglucose. The lipopolysaccharide fraction included an acidic glucomannan, a rhamnoglucan, and a heptoglucan containing D-*glycero*-D-*manno*-heptose and L-*glycero*-D-*manno*-heptose. Removal of mucopeptide material from the cell-wall fraction left an insoluble residue containing glucose and 2-amino-2-deoxyglucose.

An extracellular fructan produced by *Leuconostoc mesenteroides* strain C has been separated into two components by fractional precipitation of the fructan acetate.[132] Deacetylation of the fractions produced two fructans which contained 2% and 4% of D-glucose. Structural studies indicated the presence of (2 → 6)-linked β-D-fructofuranosyl residues with branches at C-1 and an average chain length of 5–6. The fructan precipitated specifically concanavalin A.

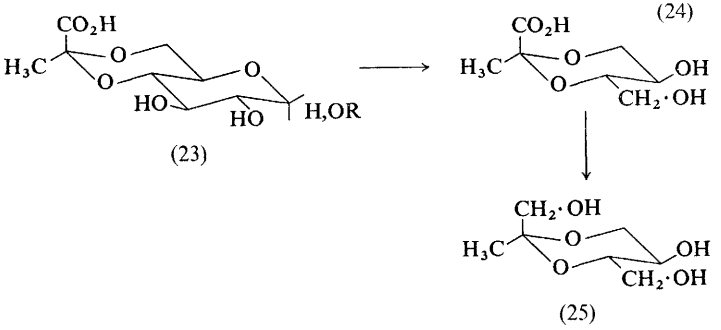
An exocellular pentosylmannan produced by the yeast *Trichosporum cutaneum* comprised an α-(1 → 3)-linked mannan backbone structure in which some of the residues were substituted with residues of D-xylopyranose, D-mannopyranose, and 4-*O*-(α-L-arabinopyranosyl)-D-xylopyranose.[133]

An exocellular glycan produced by *Myxobacterium* 402 contained[134] D-glucose (14), D-mannose (1), D-rhamnose (6), 3-*O*-methyl-D-rhamnose (5), 2-*O*-methyl-D-rhamnose (5), and 2-amino-2-deoxy-D-glucose (1). Extracellular polysaccharides of 10 strains of *Rhizobium meliloti* liberated glucose (82–86%), galactose (13–16%), and glucuronic acid (0·4–1·2%) on hydrolysis.[135]

The configuration of 4,6-*O*-(1-carboxyethylidene)-D-glucopyranosyl end units (23) present in the exocellular polysaccharide of *Xanthomonas campestris* NRRL B-1459 has been established[136] by Smith degradation to 1,3-*O*-(1-carboxyethylidene)-L-erythritol (24) and subsequent conversion to syrupy 1,3-*O*-(1-hydroxyisopropylidene)-L-erythritol whose n.m.r. spectrum and specific rotation were indistinguishable from compound (25). This,

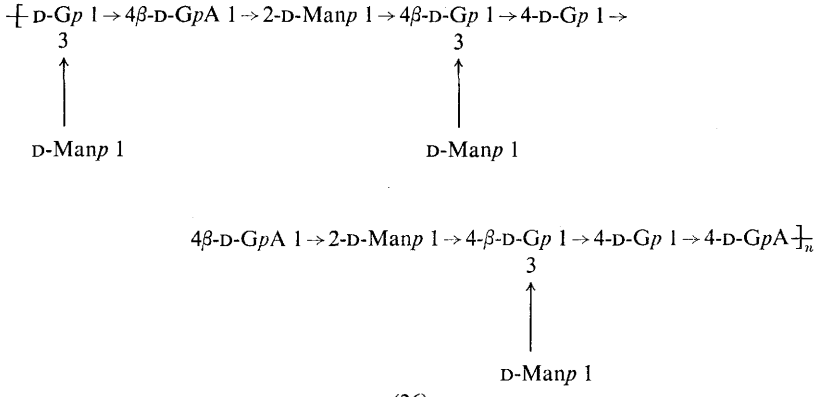
[130] A. L. Olins and R. C. Warner, *J. Biol. Chem.*, 1967, **242**, 4994.
[131] G. A. Adams and S. M. Martin, *Canad. J. Biochem.*, 1967, **45**, 477.
[132] B. A. Lewis, M. J. St. Cyr, and F. Smith, *Carbohydrate Res.*, 1967, **5**, 194.
[133] P. A. J. Gorrin and J. F. T. Spencer, *Canad. J. Chem.*, 1967, **45**, 1543.
[134] I. M. Morrison, R. Young, M. B. Perry, and G. A. Adams, *Canad. J. Chem.*, 1967, **45**, 1987.
[135] N. Amarger, M. Obaton, and H. Blachère, *Canad. J. Microbiol.*, 1967, **13**, 99.
[136] P. A. J. Gorin, T. Ishikawa, J. F. T. Spencer, and J. H. Sloneker, *Canad. J. Chem.*, 1967, **45**, 2005.

together with earlier work,[137] established that, in their most stable configuration, the 4,6-*O*-(1-carboxyethylidene)-D-glucopyranosyl units contained *C*-methyl groups equatorial to the 1,3-dioxan ring. The



(23) (24) (25)

4,6-*O*-(1-carboxyethylidene)-D-galactopyranosyl residues in agar and in a polysaccharide from *Corynebacterium insidiosum* also contained equatorial *C*-methyl groups but the acetal carbon atom had the opposite configuration.



(26)

Structure (26) was proposed[138] for an electrophoretically homogeneous, highly branched polysaccharide isolated from *Xanthomonas campestris* on the basis of methylation and earlier structural studies.[139] An electrophoretically homogeneous polysaccharide from *Arthrobacter viscosus* contained[140] D-glucose, D-mannose, and D-mannuronic acid (1 : 1 : 1·14).

[137] P. A. J. Gorin and T. Ishikawa, *Canad. J. Chem.*, 1967, **45**, 521.
[138] I. R. Siddiqui, *Carbohydrate Res.*, 1967, **4**, 284.
[139] J. H. Sloneker, D. G. Orentas, and A. Jeanes, *Canad. J. Chem.*, 1964, **42**, 1261.
[140] I. R. Siddiqui, *Carbohydrate Res.*, 1967, **4**, 277.

Methylation studies showed that the neutral sugars and most of the D-mannuronic acid were (1 → 4)-linked and that some of the residues of uronic acid occupied adjacent positions.

A polysaccharide containing galactose, glucose, mannose, arabinose, xylose, 2-amino-2-deoxyglucose, and probably fucose has been reported [141] to take part in the characterisation of the *Leptospira* antigenic complex.

An anaphylactically active polysaccharide containing arabinose and mannose (3 : 2) has been isolated [142] from human tubercle bacilli (Aoyama B strain). Several sugar phosphates probably related to cell metabolism have been isolated [143] from *Mycobacterium tuberculosis* strain BCG and assigned the structures αα-trehalose-6,6′-di-D-mannosyl 1-phosphate, α-D-mannosyl 1-phosphate, and αα-trehalose-6-D-mannosyl 1-phosphate. In the presence of isoniazid, there was an intracellular accumulation by growing *M. tuberculosis* BCG cells of a glucan, an oligosaccharide containing glucose and glucose 6-phosphate, αα-trehalose, glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate, and a compound tentatively identified as trehalose 6-phosphate.[144] It was suggested that the action of isoniazid on mycobacteria might be partial inhibition of glycolysis.

3-*O*-(α-D-Glucopyranosyl)-αβ-trehalose has been isolated [145] from *Streptococcus faecalis*. The same compound was produced by *S. faecalis* cultured in the presence of 2-deoxy-D-galactose.[146] In addition, 3-*O*-[2-deoxy-α-D-glucopyranosyl]-αβ-trehalose and 3-*O*-(α-D-glucopyranosyl)-2-deoxy-αβ-trehalose were produced indicating that the bacteria converted 2-deoxy-D-galactose into 2-deoxy-D-glucose.

Bacterial Cell-walls

Further studies have been made of the complex molecular architecture and biosynthesis of bacterial cell-walls. The chemistry of teichoic acids was the subject of a review.[147a] One of the polymeric components of bacterial cell-walls has been identified [147b] as an insoluble peptidoglycan network with a carbohydrate moiety of β-(1 → 4)-linked, alternating residues of 2-acetamido-2-deoxy-D-glucose and *N*-acetylmuramic acid. Many of the latter residues were substituted by a peptide which in most cases was the tetrapeptide N^{α}-(L-alanyl-D-isoglutaminyl)-L-lysyl-D-alanine.[148] In *Micrococcus lysodeikticus* cell-walls, however, many of the muramyl

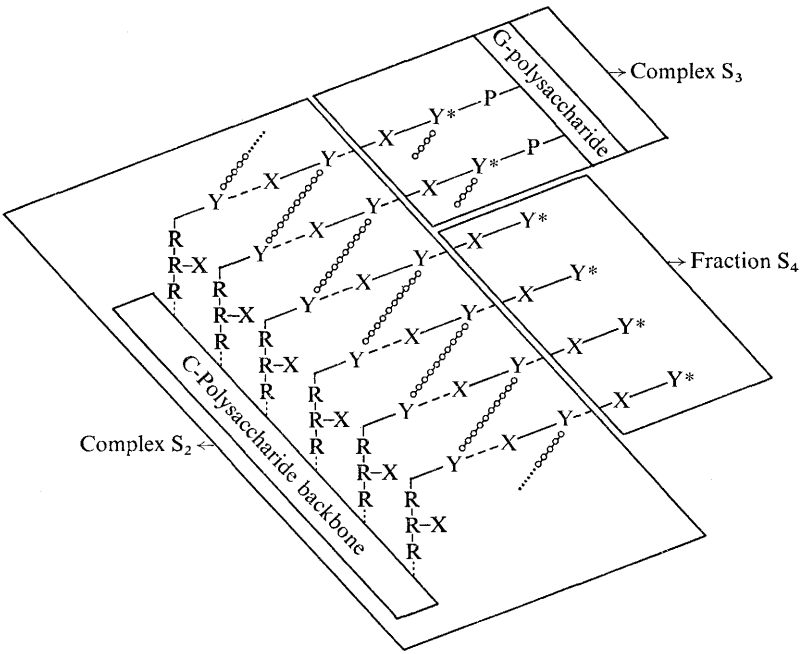
[141] A. Friedlander, E. Shenberg, S. Ben-Efraim, M. Torton, and J. van der Hoeden, *Israel J. Chem.*, 1967, **5**, 111P.
[142] I. Azuma, H. Kimura, T. Niinaka, and Y. Yamamura, *J. Bacteriol.*, 1967, **93**, 770.
[143] K. Narumi and T. Tsumita, *J. Biol. Chem.*, 1967, **242**, 2233.
[144] F. G. Winder, P. J. Brennan, and I. McDonnell, *Biochem. J.*, 1967, **104**, 385.
[145] W. Fischer and J. Krieglstein, *Z. physiol. Chem.*, 1967, **348**, 1252.
[146] J. Krieglstein and W. Fischer, *Z. physiol. Chem.*, 1967, **348**, 1256.
[147a] A. R. Archibald and J. Baddiley, *Adv. Carbohydrate Chem.*, 1966, **21**, 323.
[147b] E. Muñoz, J.-M. Ghuysen, M. Leyh-Bouille, J. F. Petit, H. Heymann, E. Bricas, and P. Lefrancier, *Biochemistry*, 1966, **5**, 3748.
[148] J.-M. Ghuysen, E. Bricas, M. Leyh-Bouille, M. Lache, and C. D. Shockman, *Biochemistry*, 1967, **6**, 2607.

residues were not substituted, and in those residues which were substituted, the α-carboxy-group of the glutamyl residue in the tetrapeptide carried a glycine residue with a free carboxy-group.[149–151] Most of the tetrapeptide residues were shown to be cross-linked either by amino-acids or by simple peptides between the *C*-terminal group of D-alanine in one tetrapeptide and the ε-amino-group of L-lysine in another. For example, in cell-walls of *Streptococcus pyogenes*, tetrapeptide units were cross-linked by L-alanyl-L-alanine residues;[152] in *S. faecalis*, by single D-isoasparaginyl residues;[148] in *Staphylococcus aureus*, by pentaglycine residues,[153] and in *Arthrobacter crystallopoietes* by L-alanyl residues.[153] Diaminopimelic acid has been reported as a cell-wall component in several bacteria.[154–156] The synthesis of N^{ε}-glycyl-N^{α}-[2-(2-acetamido-2-deoxy-3-*O*-D-glucopyranosyl)acetyl-L-alanyl-D-α-glutamyl]-L-lysyl-D-alanyl-D-alanine as a possible inhibitor of the biosynthesis of cell-wall glycopeptides has been reported.[157]

C-Polysaccharide, a second type of polymeric carbohydrate component in *S. pyogenes*, contained α-(1 → 3)-linked L-rhamnosyl residues, some of which were substituted at C-2, and terminal, nonreducing 2-acetamido-2-deoxy-D-glucosyl residues linked β-(1 → 3) to L-rhamnose.[158] Some of the 2-amino-2-deoxy-D-glucosyl residues might not be terminal. Some indication of the nature of the linkages between the various macromolecular components in the cell-walls of *S. pyogenes* was provided by the isolation and characterisation of DL-glycerol 1-phosphate, glyceryl: (1′ → 1)-L-rhamnoside 2′- (or 3′-)phosphate, and muramic acid 6-phosphate.[159] It has been proposed[159] that such units link the peptidoglycan and the C-polysaccharide. However, about 10% of the peptidoglycan units in *S. pyogenes* were linked by phosphodiester linkages to a hitherto unrecognised polysaccharide component of the cell-wall, designated as G polysaccharide.[152] The latter consisted of disaccharide-peptide monomer linked through a phosphodiester linkage to a polymer which contained, per-peptide monomer, one 2-amino-2-deoxyglucosyl residue, five to six D-glucosyl residues, and four to five unidentified hexosaminyl compounds which might be amino-hexuronic acids or related compounds. The G polysaccharide might be the moiety to which the M, R, and T protein

[149] W. Katz and J. L. Strominger, *Biochemistry*, 1967, **6**, 930.
[150] D. J. Tipper, W. Katz, J. L. Strominger, and J.-M. Ghuysen, *Biochemistry*, 1967, **6**, 921.
[151] D. Mirelman and N. Sharon, *J. Biol. Chem.*, 1967, **242**, 3414.
[152] E. Muñoz, J.-M. Ghuysen, and H. Heymann, *Biochemistry*, 1967, **6**, 3659.
[153] D. J. Tipper, J. L. Strominger, and J. C. Ensign, *Biochemistry*, 1967, **6**, 906.
[154] K. Clarke, G. W. Gray, and D. A. Reaveley, *Biochem. J.*, 1967, **105**, 749.
[155] E. Bricas, J.-M. Ghuysen, and P. Dezélée, *Biochemistry*, 1967, **6**, 2598.
[156] R. C. Hughes, *Biochem. J.*, 1967, **102**, 26P.
[157] M. C. Khosla, N. C. Chaturvedi, and N. Anand, *Indian J. Chem.*, 1967, **5**, 237.
[158] S. Estrada-Parra, M. Heidelberger, and P. A. Rebers, *J. Biol. Chem.*, 1963, **238**, 510; R. M. Krause, *Bacteriol. Rev.*, 1963, **27**, 369; H. Heymann, J. M. Manniello, and S. S. Barkulis, *J. Biol. Chem.*, 1963, **238**, 502; H. Heymann, J. M. Manniello, L. D. Zelenicke, and S. S. Barkulis, *J. Biol. Chem.*, 1964, **230**, 1656.
[159] H. Heymann, J. M. Manniello, and S. S. Barkulis, *Biochem. Biophys. Res. Comm.*, 1967, **26**, 486.

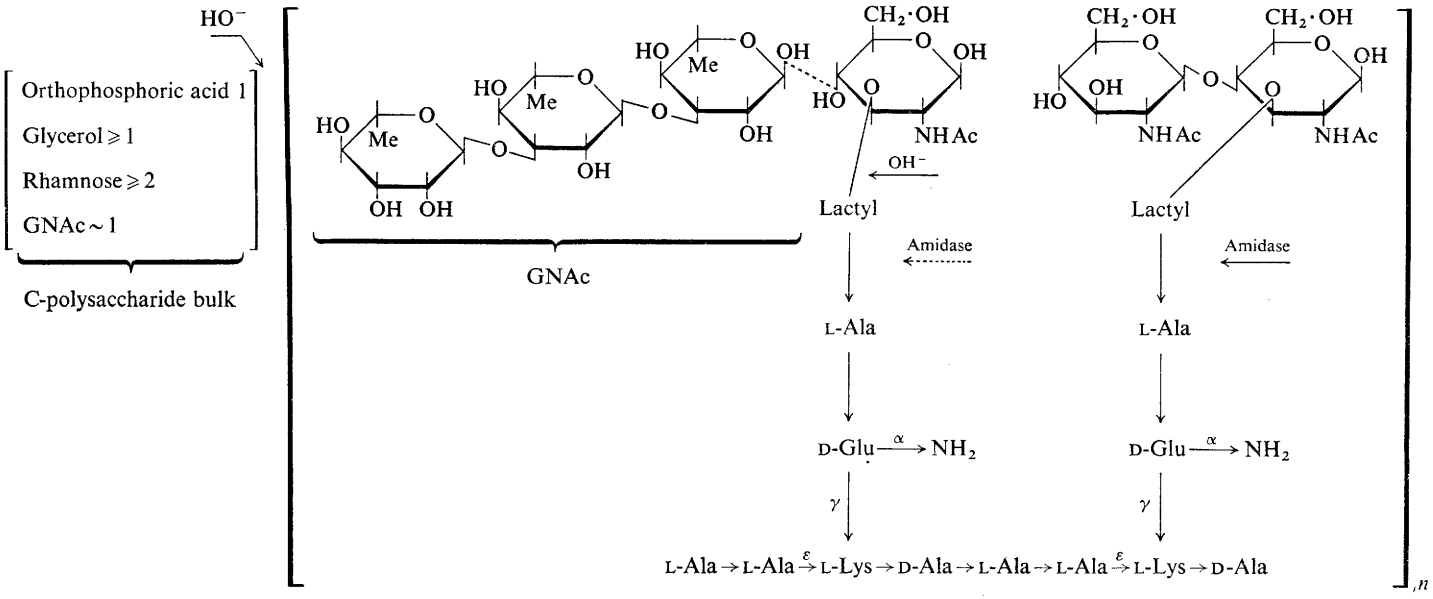
components were linked in the cell-wall. A tentative schematic structure of cell-walls of *S. pyogenes* has been proposed [152] (see 27a, b, c).



Tentative schematic structure of cell walls of *S. pyogenes*. (Only one alternative is shown.) X = 2-acetamido-2-deoxyglucose, Y = *N*-acetylmuramic acid; Y* = *N*-acetylmuramic acid at the reducing end of the glycan chains; R = L-rhamnose; P = orthophosphoric acid; (– – – –) glycosidic linkage hydrolysed by the *Streptomyces* F_1 endo-*N*-acetylmuramidase; (o–o–o–o) uncross-linked tetrapeptides; (o–o–o–o–o–o–o–o–o–o) decapeptide bridges resulting from the cross-linking of two tetrapeptides through L-Ala-L-Ala dipeptides. Hydrolysis by the F_1 enzyme liberates polymer S_2, polymer S_3, and disaccharide units in yields close to those experimentally found.

(27a)

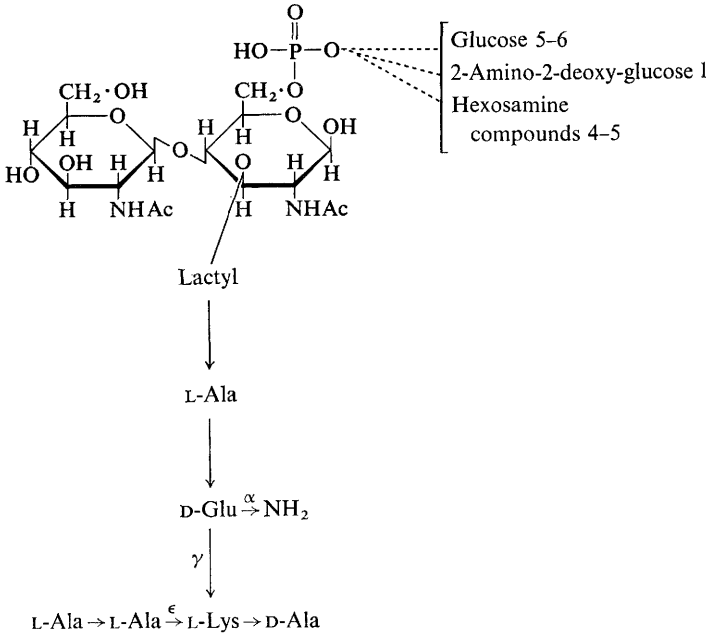
The primary site of attack of a bacteriolytic peptidase from *Myxobacter* which solubilised cell-walls of *S. aureus* and *A. crystallopoietes* was the point of attachment of the cross-linking pentaglycyl or L-alanyl units to the D-alanyl residue of the tetrapeptide.[153] The *N*-acetylmuramyl L-alanine linkages in the peptidoglycan were cleaved more slowly by the enzyme, with the release of peptide fragments, a polysaccharide–teichoic acid complex, and an undegraded, polydisperse, polysaccharide containing the same disaccharide repeating unit that was found in the peptidoglycan. The undegraded polysaccharide backbone of the peptidoglycans isolated in this way from the cell-walls of the sphere forms of *A. crystallopoietes* was heterogeneous in size, and average less than 40 hexosaminyl residues per



Fraction S_2

Proposed structure for the major polymer present in fraction S_2. A dimeric peptide unit is substituted at one of the amino-termini by a 2-acetamido-2-deoxy-β-glucosyl $(1 \rightarrow 4)$-*N*-acetylmuramic acid disaccharide and at the other terminus by an *N*-acetylmuramic acid residue glycosidically linked to a trirhamnosyl fragment of the C polysaccharide. One 2-acetamido-2-deoxyglucose residue GNAc is attached by a β-glycosidic bond to the trirhamnosyl block. Phosphate groups occur within the inner portions of the C-polysaccharide moiety.

(27b)



Fraction S_3

Proposed structure for the main complex present in fraction S_3. A disaccharide peptide monomer is linked through a phosphodiester bridge to a polysaccharide composed of glucose, 2-amino-2-deoxyglucose, and other unidentified hexosamine compounds (G polysaccharide).

(27c)

(Figures 27a–c are reproduced by permission from *Biochemistry* 1967, **6**, 3659)

chain, whereas those isolated from the cell-walls of the rod form of the same bacterium were homogeneous in size and average 114–135 hexosaminyl residues per chain.[160]

The soluble mucopeptide fraction obtained[161] by treatment of acid-extracted cell-walls of *Bacillus lichenformis* NCTC 6346 with lysozyme has been fractionated[162] according to molecular size using Sephadex G25 and G50. Approximately 50% of the fraction had M_W less than 2000 and 16% had M_W greater than 20,000. The extent of cross-linking was relatively low even in the mucopeptides of larger molecular size. It has been postulated[162] that the presence of residual phosphorus in the original acid-extracted cell-walls might have prevented a complete cleavage of glycosidic linkages that

[160] T. A. Krulwich, J. C. Ensign, D. J. Tipper, and J. L. Strominger, *J. Bacteriol.*, 1967, **94**, 734.
[161] R. C. Hughes, *Biochem. J.*, 1968, **106**, 41.
[162] R. C. Hughes, *Biochem. J.*, 1968, **106**, 49.

were potentially susceptible to lysozyme.[163] A major component of the small molecular size mucopeptide contained 2-amino-2-deoxyglucose, *N*-acetylmuramic acid, L-alanine, glutamic acid, and diaminopimelic acid, and a second fragment comprised two such molecules linked together through a molecule of D-alanine.

Glycerol has been demonstrated as a cell-wall component of *Leuconstoc citrovorum* 8082 and *L. mesenteroides* 10830a and 11449.[164] In addition the cell-walls contained glucose, rhamnose, 2-amino-2-deoxyglucose, muramic acid, and the amino-acids alanine, glutamic acid, and lysine. L-Serine was an additional major cell-wall component in *L. mesenteroides* 10830a. The chemical composition of the cell-wall of *Rikettsia mooseri* resembled that of gram-negative bacterial cell-walls more closely than that of gram-positive bacterial cell-walls.[165] Glucose, galactose, glucuronic acid, and muramic acid were demonstrated together with at least fifteen amino-acids, including diaminopimelic acid.

Further evidence for the structure of bacterial cell-walls has been provided by studies of the biosynthesis of the peptidoglycan component. 2-Amino-2-deoxyglucose was the precursor of the C1–C6 moiety of muramic acid in the cell wall of *S. pyogenes*[166] and phospholipid intermediates were involved in the biosynthesis of a peptidoglycan from UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine (UDP-*N*-acetylmuramic acid pentapeptide) and UDP-2-acetamido-2-deoxy-D-glucose with particulate enzyme from *S. aureus* and *M. lysodeikticus*.[167] The enzyme preparation from the latter organism has been separated into its protein and lipid components.[168] Disaccharide (-pentapeptide-) phospholipid intermediates from the two bacteria both contained glutamic acid, lysine, alanine, 2-acetamido-2-deoxy-glucose, and *N*-acetylmuramic acid in the molar proportions 1 : 1 : 3 : 1 : 1. The utilisation of this intermediate for peptidoglycan synthesis was inhibited by low concentrations of ristocetin and vancomycin.[167] The same intermediate accepted glycine from glycyl-soluble ribonucleic acid in the biosynthesis of the pentaglycine chains of

[163] Studies of the structure and specificity of lysozyme have been described in the following refs.: C. C. F. Blake, L. N. Johnson, G. A. Mair, A. T. C. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc.*, 1967, *B*, **167**, 387; D. M. Chipman, V. Grisaro, and N. Sharon, *J. Biol. Chem.*, 1967, **242**, 4388; F. W. Dahlquist and M. Raferty, *Nature*, 1967, **213**, 625; S. Hara and Y. Matsushima, *J. Biochem.*, 1967, **62**, 118; J. Jollès and P. Jollès, *Biochemistry*, 1967, **6**, 411; G. Lowe, G. Sheppard, M. L. Sinnott, and A. Williams, *Biochem. J.*, 1967, **104**, 893; A. Neuberger and B. M. Wilson, *Biochim. Biophys. Acta*, 1967, **147**, 473; *Nature*, 1967, **215**, 524; J. J. Père, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 365; D. C. Phillips, *Proc. Natl. Acad. Sci., U.S.A.*, 1967, **57**, 484; J. J. Pollock, D. M. Chipman, and N. Sharon, *Arch. Biochem. Biophys.*, 1967, **120**, 235; *Biochem. Biophys. Res. Comm.*, 1967, **28**, 779; J. A. Rupley and V. Gates, *Proc. Natl. Acad. Sci., U.S.A.*, 1967, **57**, 496.

[164] S. J. Harney, N. D. Simopoulos, and M. Ikawa, *J. Bacteriol.*, 1967, **93**, 273.

[165] W. H. Wood and C. L. Wisseman, jun., *J. Bacteriol.*, 1967, **93**, 1113.

[166] S. S. Barkulis, J. J. Boltralik, H. Hankin, and H. Heymann, *J. Bacteriol.*, 1967, **94**, 963.

[167] J. S. Anderson, M. Matsuhashi, M. A. Haskin, and J. L. Strominger, *J. Biol. Chem.*, 1967, **242**, 3180.

[168] C. P. Dietrich, A. V. Colucci, and J. L. Strominger, *J. Biol. Chem.*, 1967, **242**, 3218.

the peptidoglycan of *S. aureus*,[169] whereas the addition of the single glycine residue to the α-carboxy-group of glutamic acid in the peptidoglycan of *M. lysodeikticus* occurred by a different mechanism which did not require soluble ribonucleic acid.[170]

L-Forms of *S. aureus* do not synthesise cell-wall mucopeptide. Given the appropriate substrates, however, they synthesised lipid intermediates and peptidoglycan, although they could not complete the synthesis of cross-linked peptidoglycan.[171] Chemical comparisons of the membranes of protoplasts and L-forms of *S. aureus* showed[172] that the L-forms contained more glycolipid than the parent bacteria. In all membranes the glycolipid, which accounted for all the carbohydrate present, was identified as di-D-glucosyldiglyceride.

Fungal Polysaccharides

Further structural studies have been reported of polysaccharides isolated from a range of fungi and in some cases the biological properties of such materials have been investigated.

A water-soluble polysaccharide isolated from the fruit bodies of *Armillaria mellea* contained D-galactose, D-mannose, and L-fucose (6·5 : 1 : 2). A backbone structure of α-(1 → 6)-linked D-galactopyranosyl residues was proposed[173] in which approximately every third residue was substituted at C-2 by either L-fucopyranosyl or 3-*O*-(α-D-mannopyranosyl)-L-fucopyranosyl residues. A fucomannogalactan with essentially the same structure has been isolated[174] from fruit bodies of *Polyporus pinicola*. A complex mixture of polysaccharides present in aqueous extracts of the mycelium of *A. mellea* grown on *D*-glucitol as the sole carbon source[175] included a xylomannan, with an α-(1 → 3)-linked mannan backbone in which every second or third residue was substituted at C-4 by a 4-*O*-(α-D-xylopyranosyl)-D-xylopyranosyl residue, and a polysaccharide or polysaccharides in which chains of α-(1 → 6)-linked D-galactopyranosyl residues were substituted at C-2 by L-fucopyranosyl residues or by 3-*O*-(α-D-mannopyranosyl)-L-fucopyranosyl and 6-*O*-(3-*O*-methyl-α-D-galactopyranosyl)-D-galactosyl residues. A fucoxylomannan, unrelated to the xylomannan from *A. mellea*, has been isolated[174] from the fruit bodies of *P. pinicola*. Structural features of the polysaccharide included D-mannosyl units, present both as chain units and as branch points, (1 → 2)-linked and terminal, nonreducing D-xylosyl residues, and L-fucosyl residues present solely as nonreducing end groups. The glycosidic linkages probably had the β-configuration.

[169] M. Matsuhashi, C. P. Dietrich, and J. L. Strominger, *J. Biol. Chem.*, 1967, **242**, 3191.
[170] W. Katz, M. Matsuhashi, C. P. Dietrich, and J. L. Strominger, *J. Biol. Chem.*, 1967, **242**, 3207.
[171] A. N. Chatterjee, J. B. Ward, and H. R. Perkins, *Nature*, 1967, **214**, 1311.
[172] J. B. Ward and H. R. Perkins, *Biochem. J.*, 1968, **106**, 391.
[173] R. N. Fraser and B. Lindberg, *Carbohydrate Res.*, 1967, **4**, 12.
[174] R. N. Fraser, S. Karácsonyi, and B. Lindberg, *Acta Chem. Scand.*, 1967, **21**, 1783.
[175] H. O. Bouveng, R. N. Fraser, and B. Lindberg, *Carbohydrate Res.*, 1967, **4**, 20.

Structurally similar glucans have been isolated [176] from *Candida albicans* (serotype B) and from *C. parapsilosis*. The major structural feature in each glucan was a chain of (1 → 6)-linked D-glucopyranosyl residues, with a smaller number of (1 → 3) linkages in the linear portion of the polysaccharide. In the glucan from *C. albicans* branching occurred exclusively at C-3 and C-6, whereas in the glucan from *C. parapsilosis* branching occurred at C-3 and C-6 of some glucosyl residues, and at C-4 and C-6 of others. Similar glucans have been found in *C. albicans* (serotype A) in *Saccharomyces cerevisiae* and in a series of dermatophytes.

Investigations of certain oomycetous fungi showed that the cell-walls were composed primarily of non-cellulosic glucans containing β-(1 → 3) and β-(1 → 6) D-glucosidic linkages.[177] Similar conclusions could be made from studies [178] of the degradation of isolated hyphal walls of *Phytophthora cinnamorii* by a streptococcal enzyme preparation, containing cellulase and small amounts of other glucanases. The products included glucose, cellobiose, laminaribiose, gentiobiose, and soluble, nondialysable glucose polymers. Similar disaccharides were produced by partial acid hydrolysis of the cell-walls. Isolated cell-walls from *Phytophthora heveae*, *Pythium butleri*, and *Saprolegnia ferax* contained 80–90% carbohydrate of which 30–45% was identified as cellulose.[179] Galactose, mannose, rhamnose, and ribose in addition to glucose were monosaccharide components of the cell-walls.

Methylation and periodate oxidation studies of mannans isolated from *Saccharomyces cerevisiae* indicated the presence of (1 → 2)- and (1 → 6)-linked-D-mannopyranosyl residues.[180] Chemical studies [181] of serologically similar mannans from *Candida albicans* (serotypes A and B), *C. parapsilosis*, *C. stellatoidea*, and *C. tropicalis* showed a predominance of (1 → 2) linkages in the linear portions with smaller amounts of (1 → 6) and (1 → 3) linkages. Some of the D-mannopyranosyl and D-mannofuranosyl units were branched at C-2 and C-6, and the branches were terminated by D-mannopyranosyl units. Most of the glycosidic linkages had the α-configuration. Immunochemically different mannans extracted from *C. albicans* and *S. cerevisiae* by autoclaving at pH 7 have been separated on DEAE-Sephadex into acid and neutral components.[182]

Rabbit antisera to the mycelium of the dermatophyte *Microsporum quinckeanum* reacted with three neutral polysaccharides (glucan, galactomannans I and II) isolated from each of five species of dermatophytes (*M. quinckeanum*, *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, and

[176] R. J. Yu, C. T. Bishop, F. P. Cooper, F. Blank, and H. F. Hasenclever, *Canad. J. Chem.*, 1967, **45**, 2264.
[177] J. M. Aronson, B. A. Cooper, and M. S. Fuller, *Science*, 1967, **155**, 332.
[178] S. Bartnicki-Garcia and E. Lippman, *Biochim. Biophys. Acta*, 1967, **136**, 533.
[179] M. Novaes-Ledieu, A. Jimenez-Martinez, and J. R. Villanueva, *J. Gen. Microbiol.*, 1967, **47**, 237.
[180] N. K. Kochetkov, O. S. Chishov, and A. I. Usov, *Zhur. obshchei. Khim.*, 1967, **37**, 91.
[181] R. J. Yu, C. T. Bishop, F. P. Cooper, H. F. Hasenclever, and F. Blank, *Canad. J. Chem.*, 1967, **45**, 2205.
[182] O. Sakaguchi, S. Suzuki, M. Suzuki, and H. Sunayama, *Jap. J. Microbiol.*, 1967, **11**, 119.

T. schoenleinii.[183] Significant differences were observed among the galactomannans II and the glucans, but not among the galactomannans I.

Two structurally similar galactomannanpeptides purified from the culture filtrate and dried cells, respectively, of *Aspergillus fumigatus*, both contained equimolar amounts of galactose and mannose.[184] Hydrolysis, methylation, and Smith degradation studies indicated a structure in which a mannan core with α-(1 → 2), α-(1 → 3), and α-(1 → 6)-linked residues was substituted with side-chains of galactofuranose and an oligosaccharide containing galactose and mannose (4 : 5).

A mixture of galactomannan peptides extracted with ethylene glycol from the mycelium of *Trichophyton mentagrophytes* has been purified[185] by precipitation, with a detergent, of their complexes with borate ion, and subsequent gel and ion-exchange chromatography. The galactomannan peptides elicited delayed- and immediate-type hypersensitivity reactions in guinea pigs sensitised with whole mycelium or with purified galactomannan peptides. The immediate-type reactivity was associated with the carbohydrate moiety of the glycopeptide, although selective removal of the D-galactofuranosyl residues did not alter immediate- or delayed-type activities, and the delayed-type activity was associated with the peptide component. Thus, those galactomannan peptides which contained more protein gave greater delayed-type activity. The results of heterologous and homologous immediate- and delayed-type hypersensitivity tests with purified galactomannan peptides from several dermatophytes emphasised the close structural similarities of such products not only within a genus but also between different genera of dermatophytes.[186] Certain bacterial polysaccharides gave good immediate-type reactions in dermatophyte-sensitised animals and possessed structural features similar to those reported for the carbohydrate moiety of allergenic glycopeptides from dermatophytes, and to those of pure galactomannans isolated from the mycelia by more drastic methods. Thus, those polysaccharides which gave good immediate-type reactions were mostly highly branched and contained (1 → 2), (1 → 3), and (1 → 6)-linked D-mannopyranose residues as well as (1 → 2, 6 ← 1) branch units and terminal nonreducing residues, with a preponderance of α-glycosidic linkages. A galactomannan, containing galactose and mannose in the ratio 1 : 2·24, has been isolated by other workers[187] from the culture medium of *T. rubrum*, which also contained components, presumably glycopeptides, which contained mannose, galactose, glucose, and xylose.

A glycopeptide, containing glucose, galactose, arabinose, and 2-amino-2-deoxyglucose, has been isolated[188] both from the mycelial and from

[183] S. F. Grappel, F. Blank, and C. T. Bishop, *J. Bacteriol.*, 1967, **93**, 1001.
[184] O. Sakaguchi, K. Yokata, and M. Suzuki, *Yakugaku Zasshi*, 1967, **87**, 1268.
[185] S. A. Barker, O. Basarab, and C. N. D. Cruickshank, *Carbohydrate Res.*, 1967, **3**, 325.
[186] O. Basarab, C. N. D. Cruickshank, and M. J. How, *Sabouraudia*, 1968, **6**, 119.
[187] S. Suzuki, M. Suzuki, and O. Sakaguchi, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 976.
[188] A. Restrepo-Moreno and J. D. Schneidau, jun., *J. Bacteriol.*, 1967, **93**, 1741.

yeast-phase culture filtrates of *Paracoccidioides brasiliensis*. Both preparations elicited skin reactions in infected guinea pigs and humans.

Cell-wall preparations from the yeast-like phase of *Histoplasma capsulatum* and *Saccharomyces cerevisiae* contained much larger quantities of chitin and smaller amounts of mannose and amino-acids than did the cell-walls of the mycelial phase.[189] The biosynthesis of chitin in the spores and growing cells of the aquatic fungus *Blastocladiella emersonii* has been investigated.[190] Although the spores lacked the chitinous cell-wall present in the cellular form, chitin synthetase activity has been demonstrated both in spores and cells. Di-*N*-acetylchitobiose was an intermediate in the biosynthesis of chitin.

The soluble polysaccharide from culture filtrates of *Cryptococcus neoformans* was rendered potently antigenic in mice if chemically coupled with bovine γ-globulin with retention of the acetyl groups of the native polysaccharide.[191] Coupling was effected by controlled nitrocarbanilation of the polysaccharide in DMSO followed by sequential reduction, diazotisation, and reaction with protein.

[189] J. E. Domer, J. G. Hamilton, and J. C. Harkin, *J. Bacteriol.*, 1967, **74**, 466.
[190] E. P. Camargo, C. P. Dietrich, D. Sonneborn, and J. L. Strominger, *J. Biol. Chem.*, 1967, **242**, 3121.
[191] M. B. Goren and G. M. Middlebrook, *J. Immunol.*, 1967, **98**, 901.

5
Glycoproteins and Glycopeptides

A review[192] of the structure and function of some of the better known mammalian glycoproteins included references to work published in 1966.

Carbohydrate–peptide Linkages

The structural importance of *O*-glycosylated hydroxy-amino-acids in glycoproteins and sulphated glycosaminoglycans has prompted a detailed study[193] of the properties of D-glucopyranosyl-, D-galactopyranosyl-, and D-galactofuranosyl-L-serine. The hexopyranosyl serines were synthesised by Koenigs–Knorr glycosylation of *N*-benzyloxycarbonyl-L-serine methyl ester and subsequent deacetylation with triethylamine at room temperature, and removal of the *N*-benzyloxycarbonyl group by hydrogenolysis. *N*-Benzyloxycarbonyl-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-L-serine methyl ester was synthesised by glycosylation of *N*-benzyloxycarbonyl-L-serine methyl ester with 3,5,6-tri-*O*-acetyl-α-D-galactofuranose 1,2-(methyl orthoacetate). Deacetylation and hydrogenolysis gave D-galactofuranosyl-L-serine methyl ester.

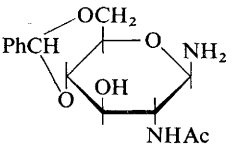
Glycosidic bonds in the serine glycosides showed acid-stability typical of that expected for glycosides. Substitution of the amino-group of the serine moiety increased the stability of the glycosidic bond. In alkali the serine glycosides underwent β-elimination with the formation of monosaccharides and derivatives of α-amino-acrylic acid and the alkaline-stability of the *O*-glycosides of *N*-benzyloxycarbonyl-L-serine increased in the series methyl ester < methylamide < acid. At pH 11, cleavage of the glycosidic bond of the methyl ester derivatives rapidly ceased since the competing hydrolysis of the methyl ester predominated to give an acid in which the glycosidic bond was more stable to alkaline cleavage. However, at pH 8–9 the competing ester hydrolysis was less important. The alkaline stability of the glycosidic bond was influenced by substitution of the amino-group and increased in the series *N*-benzyloxycarbonyl < *N*-benzyloxycarbonyl-*N*-glycyl < *N*-glycyl ≤ amino.

O-(β-D-Glucopyranosyl)-L-serine was stable to 0·1 N-sodium hydroxide at 20° for 1 week.[193] Studies of the action of alkali on *O*-seryl and *O*-threonyl

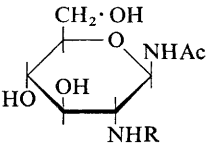
[192] P. W. Kent, *Essays in Biochemistry*, 1967, 3, 105.
[193] V. A. Derevitskaya, M. G. Vafina, and N. K. Kochetkov, *Carbohydrate Res.*, 1967, 3, 377.

glycosides of 2-acetamido-2-deoxy-β-D-glucose showed[194] that the glycosidic bond was cleaved only if the carboxy-group of the hydroxy-amino-acid was substituted. The corresponding amides, however, were cleaved by alkaline borohydride with the destruction of serine and threonine. The structure of the monosaccharide unit in serine glycosides had little influence on the alkali-stability of the glycosidic bond.

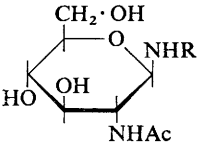
Another type of glycopeptide bond in glycoproteins involves an amido-linkage between the β-carboxy-group of aspartic acid and the amino-group of 2-acetamido-2-deoxy-β-D-glucopyranosylamine. A series of *N'*-acyl-2-acylamido-2-deoxy-β-D-glucopyranosylamines has been synthesised[195] by reaction of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine (28) with a suitably protected amino-acid in the presence of DCC followed by the removal of protecting groups. Synthetic *N'*-acetyl-2-[(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine (29) showed identical



(28)



(29)



(30)

R = β-L-aspartyl

chromatographic and electrophoretic properties with 2-acetamido-1-[*N*-(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine (30) and with the 2-acetamido-2-deoxy-D-glucose-L-aspartic acid compound, reported to be identical with (30), isolated from hen-egg albumen. The possibility that the latter compound possessed structure (29) was eliminated, however, by determining the rates of hydrolysis of (29) and (30).[196] The rates of hydrolysis of *N*-acetyl or *N*-aspartyl groups at C-1 were much higher than those of the corresponding groups at C-2.

The hydrolysis of *O*-seryl glycosides of 2-acetamido-2-deoxy-D-galactose present in glycopeptides prepared from sialic acid-free ovine submaxillary glycoprotein was catalysed by a glycosidase from snail (*Helix pomatia*).[197, 198] The mechanism of the cleavage was not β-elimination and the rate of cleavage increased with the molecular weight of the glycopeptide up to a certain limit.[198] An amidase, purified from rat liver and kidney, catalysed the hydrolysis of 2-acetamido-1-[*N*-(β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine.[199]

[194] J. Montreuil, M. Monsigny, and M.-T. Buchet, *Compt. rend.*, 1967, **D**, **265**, 2068.
[195] J. Yoshimura, H. Hashimoto, and H. Ando, *Carbohydrate Res.*, 1967, **5**, 82.
[196] J. Yoshimura and H. Hashimoto, *Carbohydrate Res.*, 1967, **4**, 435.
[197] A. S. Bhargava and A. Gottschalk, *Biochim. Biophys Acta*, 1967, **148**, 132.
[198] A. S. Bhargava and A. Gottschalk, *Biochim. Biophys. Acta*, 1967, **148**, 125.
[199] S. Mahadevan and A. L. Tappel, *J. Biol. Chem.*, 1967, **242**, 4568.

Plant Glycoproteins

Evidence for the presence of glycoproteins in plants was provided [200a] by the isolation of a glycopeptide in 3% yield from proteolytic digests of stem bromelain, a plant proteinase. The glycopeptide contained aspartic acid (3 mol.), serine (3), glutamic acid (2), mannose (3), xylose (1) and fucose (1) per mol. of peptide. Hydroxyproline, serine, and threonine, in nearly equimolar amounts, comprised one-third of the amino-acid residues of a protein–polysaccharide complex extracted with 15% trichloroacetic acid from corn (*Zea mays*) pericarp.[57b] The polysaccharide portion contained glucose as the predominant sugar with small amounts of galactose, arabinose, xylose, and 2-amino-2-deoxyglucose. Digestion of the complex with cellulase left a homogeneous core that contained most of hydroxyamino-acids and 2-amino-2-deoxyglucose.

Mammalian Glycoproteins

Collagen.—The presence of glycopeptide structures in soluble collagen has been indicated [200b] by the isolation from alkaline hydrolysates of guinea-pig soluble collagen of glycosides in which C-1 of glucosylgalactose or galactose was linked to the δ-hydroxy-group of hydroxylysine. Other workers also have reported [201] hexoses as integral components of bone collagen.

Bone.—Ethylenediaminetetra-acetic acid extracts of bovine cortical bone contained at least 60% of the nondialysable glycosaminoglycan uronic acid.[202] The product has been fractionated into three components (see Table 1) homogeneous in electrophoresis and ultracentrifugation.

Table 1

Component (%)	*Fraction* 1	*Fraction* 2	*Fraction* 3
N	6·2	5·5	2·7
Total amino-acids	27·46	21·76	1·80
Uronic acid	17·1	22·1	30·1
2-Amino-2-deoxygalactose	13·9	17·9	25·1
2-Amino-2-deoxyglucose	1·7	1·2	0·1
Hexose	5·2	4·2	0·9
Sialic acid	12·1	6·9	0·5
Sulphate	8·5	10·9	16·9

The amino-acid composition of fractions 1 and 2 was similar. Fraction 1 contained the neutral sugars galactose, fucose, xylose, and mannose and fraction 2 only galactose and trace amounts of xylose. It was suggested that fractions 1 and 2 contained chondroitin sulphate linked to a glycoprotein similar to the sialoprotein of cortical bone described below. Fraction 1 contained an alkali-labile glycopeptide linkage.

[200a] T. Murachi, A. Suzuki, and N. Takahashi, *Biochemistry*, 1967, **6**, 3730.
[200b] L. W. Cunningham, J. D. Ford, and J. P. Segnest, *J. Biol. Chem.*, 1967, **242**, 2570.
[201] C. J. Francis and M. Glimcher, *Experientia*, 1967, **23**, 22.
[202] G. M. Herring, *Biochem. J.*, 1967, **104**, 19P.

The homogeneous glycoprotein, M_W *ca.* 23,000, isolated from bovine cortical bone [203] contained a considerably branched carbohydrate with nonreducing residues of fucose, *N*-acetylneuraminic acid (18·4%), and *N*-glycolylneuraminic acid (2·1%). The galactosyl residues (8·2%) were mainly as the subterminal sugar in the intact glycoprotein. Other sugar components included mannose (2·5%), 2-amino-2-deoxygalactose (4·6%), and 2-amino-2-deoxyglucose (4·6%). The polypeptide chain had no detectable free terminal amino-group and was rich in aspartic and glutamic acids, serine, threonine, and glycine. A structure for the glycoprotein has been postulated in which a single large carbohydrate chain, *M* 9220, was probably joined by a glycosylamine bond to an asparagine residue of the polypeptide.

The binding of calcium, yttrium, and hydrogen ions to an acidic glycoprotein from cortical bone has been studied.[204]

Brain.—A series of sialic acid-containing glycoproteins, released from defatted rat brain by digestion with papain, were assumed to possess the same repeating unit of hexosamine and hexose but to differ in the numbers of residues of fucose and *N*-acetylneuraminic acid.[205] Most of the hexosamine was 2-amino-2-deoxyglucose and most of the hexose was accounted for as mannose and galactose with trace amounts of glucose in some fractions.

Aorta.—Structurally similar glycopeptides were isolated [206] from pronase digests of glycoproteins from the aorta of sheep, horse, and pig. All contained hexosamines, mannose, glucose, and galactose, the latter two sugars mainly occupying terminal, nonreducing ends. A disaccharide tentatively identified as 6-(*O*-mannosyl)mannose was present in partial hydrolysates.

Submaxillary Gland.—An electrophoretically and serologically homogeneous glycoprotein from human saliva which showed moderate haemagglutination inhibition against influenza and other viruses contained 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, sialic acid, and high amounts of aspartic and glutamic acids, threonine, serine, and glycine.[207] Pure cultures of human oral bacteria, capable of producing neuraminidase and enzymes which catalysed the degradation of *N*-acetyl-D-neuraminic acid and 2-acetamido-2-deoxy-D-mannose, have been isolated from saliva.[208, 209] The sequential action of such enzymes on salivary glycoproteins is probably important in the formation of dental plaque and calculus.

[203] A. T. de B. Andrews, G. M. Herring, and P. W. Kent, *Biochem. J.*, 1967, **104**, 705.
[204] A. R. Peacocke and P. A. Williams, *Biochem. J.*, 1967, **105**, 1171; P. A. Williams and A. R. Peacocke, *Biochem. J.*, 1967, **105**, 1177.
[205] E. G. Brunngraber and B. D. Brown, *Biochem. J.*, 1967, **102**, 16P; 1967, **103**, 65, 73.
[206] M. Moczar, E. Moczar, and L. Robert, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 380.
[207] G. Rølla and J. Johnsen, *Acta Path. Microbiol. Scand.*, 1967, **69**, Suppl. 187, 96.
[208] S. A. Leach and M. L. Hayes, *Nature*, 1967, **216**, 599.
[209] M. J. How, V. J. W. Long, and S. M. Woodbury, *Nature*, 1967, **214**, 1249.

Alkaline treatment (0·1 M-sodium hydroxide at 100°) of sialic acid-free ovine submaxillary gland glycoprotein released 98% of the 2-acetamido-2-deoxygalactosyl residues in 1 hr. with a concomitant loss of nearly equimolar amounts of serine and threonine.[210] These results indicated that at least 98% of the disaccharide residues *N*-acetylneuraminyl-(2 → 6)-2-acetamido-2-deoxygalactose were linked *O*-glycosidically to seryl and threonyl residues in the polypeptide chain. Approximately 8% of the *O*-glycosidic linkages to serine and threonine in the sialic acid-free glycoprotein and 19% in the native glycoprotein were less alkali-labile due probably to the presence of a negatively charged group of a glutamyl or aspartyl residue, in the vicinity of the glycosidic bond. In the native ovine glycoprotein, aspartic and glutamic acids comprised 14% of its amino-acid residues, some of which were liberated on preparation of the sialic acid-free glycoprotein by acid hydrolysis. Treatment of bovine and ovine submaxillary gland glycoproteins with alkaline borohydride at 45° for 10 hr. converted essentially all the carbohydrate chains to dialysable material.[211] However, 17–25% of the hexosamine was not converted to hexosaminitol by such treatment and was probably present as a glycopeptide. All the sialic acid residues of bovine submaxillary glycoprotein could be liberated by neuraminidase only after prior removal of *O*-acetyl groups.

Further evidence for a glycopeptide linkage involving 2-acetamido-2-deoxy-D-galactose and threonyl and/or seryl residues in ovine submaxillary gland was provided by biosynthesis.[212] A particulate enzyme from ovine submaxillary gland catalysed the transfer of 2-acetamido-2-deoxy-D-galactose from its UDP derivative only to protein acceptors prepared by enzymic or chemical treatments of ovine submaxillary glycoprotein that removed the disaccharide residues. The 2-acetamido-2-deoxygalactosyl linkages produced were alkali-labile.

Serum Glycoproteins.—Recent knowledge of the structure and metabolism of blood glycoproteins has been reviewed.[213] A new, mild method for the isolation of serum glycoproteins, based on their electrostatic interaction with chondroitin sulphates, has been proposed.[214]

Structural studies of I_gA immunoglobulin have demonstrated[215] the presence of two types of glycopeptide linkage in the same glycoprotein. The majority of glycopeptides isolated from a homogeneous A myeloma globulin type K had oligosaccharide units containing L-fucose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and *N*-acetyl-D-neuraminic acid attached to the protein by an *N*-glycosidic linkage involving hexosamine and the amide group of L-asparagine.[215, 216] Other glycopeptides in which

[210] A. S. Bhargava and A. Gottschalk, *Biochim. Biophys. Acta*, 1967, **148**, 132.
[211] M. Bertolini and W. Pigman, *J. Biol. Chem.*, 1967, **242**, 3776.
[212] E. J. McGuire and S. Roseman, *J. Biol. Chem.*, 1967, **242**, 3745.
[213] J. Musil, *Chem. listy*, 1967, **61**, 58.
[214] A. J. Anderson, *Biochem. J.*, 1967, **102**, 719.
[215] G. Dawson and J. R. Clamp, *Biochem. Biophys. Res. Comm.*, 1967, **26**, 349.
[216] J. R. Clamp, G. Dawson, and L. Hough, *Biochem. J.*, 1966, **100**, 35C.

the carbohydrate moiety contained only D-galactose and 2-acetamido-2-deoxy-D-galactose were also present and in the latter type 2-acetamido-2-deoxy-D-galactosyl residues were linked *O*-glycosidically to serine.[215, 217] An oligosaccharide unit, containing D-galactose and 2-acetamido-2-deoxy-D-galactose as the sole sugars, attached to the heavy chain of an I_gA myeloma globulin in the vicinity of an interchain disulphide bond has been reported.[218] Comparative studies of a type K and a type L I_gA immunoglobulin showed[219] that the type K glycoproteins appeared to contain three oligosaccharide units whereas the type L had three or more units. The content of L-fucose and *N*-acetylneuraminic acid and the ratio of D-mannose to D-galactose were different for the two immunoglobulins. Type K might possess 11–12 nonreducing end groups, made up of L-fucosyl and neuraminyl residues, and 7–8 residues of *N*-acetyl-D-glucosamine.

Later work showed[220] that glycopeptides derived from apparently homogeneous plasma glycoproteins exhibited two types of heterogeneity, designated as central and peripheral heterogeneity. Glycopeptides of the former type possessed oligosaccharide units in which the ratios and, in some cases, the types of monosaccharide unit were completely dissimilar, whereas the oligosaccharide units of glycopeptides which showed peripheral heterogeneity had similar carbohydrate compositions, but varied in their content of certain terminal monosaccharides. I_gA Myeloma globulin gave three different types of glycopeptides that showed central heterogeneity. In the first type the core contained D-galactose and 2-amino-2-deoxy-D-galactose (3 : 3), in the second, D-mannose and 2-amino-2-deoxy-D-glucose (4 : 3) and in the third, D-mannose, D-galactose, and 2-amino-2-deoxy-D-glucose (3 : 2 : 3). The latter core units also showed peripheral heterogeneity in the attachment of L-fucose, 2-amino-2-deoxy-D-glucose, and *N*-acetyl-D-neuraminic acid. Both types of heterogeneity were also shown by oligosaccharide units of an I_gM immunoglobulin in which one core unit contained D-mannose, D-galactose, and 2-amino-2-deoxy-D-glucose (3 : 2 : 3) which exhibited peripheral heterogeneity as in the corresponding I_gA oligosaccharide. A second core unit from the I_gM contained D-mannose and 2-amino-2-deoxy-D-glucose in ratios varying from 2 : 1 to 4 : 1.

A glycopeptide isolated from a mercuripapain digest of an immunoglobulin secreted by a plasma cell tumour in mice contained galactose, mannose, 2-amino-2-deoxyglucose, and small amounts of fucose together with aspartic and glutamic acids, serine, glycine, and isoleucine.[221] Carbohydrate and peptide were probably linked by 2-acetamido-1-(β-L-asparta-mido)-1,2-dideoxy-β-D-glucose. The effects of periodate oxidation,

[217] G. Dawson and J. R. Clamp, *Biochem. J.*, 1967, **103**, 5P.
[218] A. Ko, J. R. Clamp, G. Dawson, and J. Cebra, *Biochem. J.*, 1967, **105**, 35P.
[219] J. R. Clamp and F. W. Putnam, *Biochem. J.*, 1967, **103**, 225.
[220] J. R. Clamp, G. Dawson, and B. P. Spragg, *Biochem. J.*, 1968, **106**, 16P.
[221] T. J. Coleman, R. D. Marshall, and M. Potter, *Biochim. Biophys. Acta*, 1967, **147**, 396.

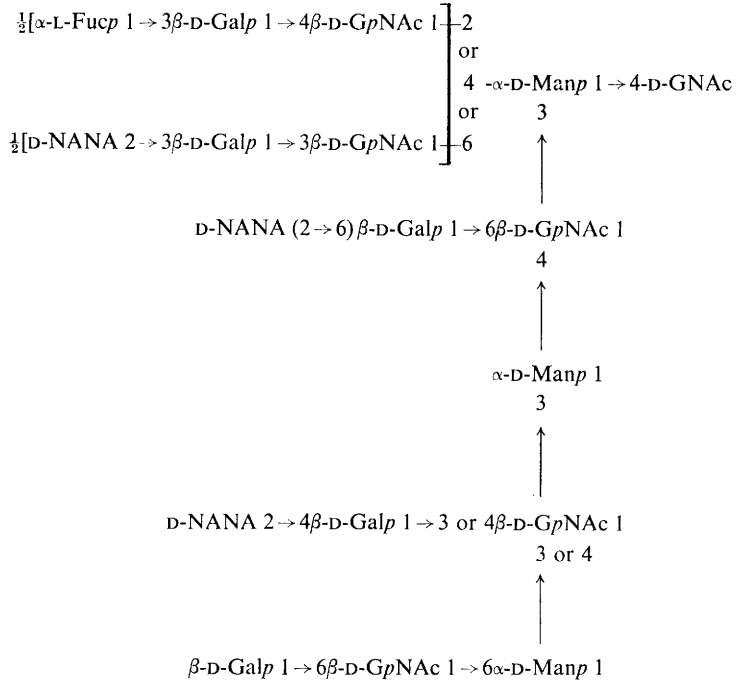
iodination, and removal of sialic acid residues on the biological and immunological activities of sheep plasma glycoprotein have been studied.[222] Removal of sialic acid gave a product which was more susceptible to proteolytic enzymes but which reacted with anti-sheep plasma protein serum. Destruction of the sialic acid and fucose and one-third of the hexose by periodate oxidation gave a product which did not cross-react with antiserum, whereas iodination did not alter the immunological cross-reactivity.

Comparative chemical studies [223] of electrophoretically homogeneous glycoproteins from bovine, porcine, and avian plasmas showed pronounced species variations in the content of fucose, but not of hexosamine, and in the amounts of certain amino-acids.

Treatment of human erythrocytes with trypsin released glycopeptides which contained between one-third and one-half of the sialic acid of the erythrocytes.[224] The glycopeptides, all of which had a molecular weight of approximately 10,000, were rich in serine and threonine and contained galactose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and *N*-acetylneuraminic acid. Some of the glycopeptide bonds were alkali-stable but part of the carbohydrate moiety was present as relatively small oligosaccharides linked to peptide through alkali-labile *O*-glycosidic bonds involving 2-acetamido-2-deoxygalactose and the hydroxy-groups of serine or threonine.

α_1- and α_2- Acid Glycoproteins.—A possible average structure (31) for the carbohydrate moiety isolated after hydrazinolysis of the α_1-acid glycoprotein (orosomucoid) from pooled human plasma has been postulated [225] from the results of oxidation with periodate and sequential oxidation with periodate, reduction with borohydride, and hydrolysis with acid (Smith degradation). A discrepancy between these results and those of other workers [226] was the higher percentage of periodate-resistant D-mannosyl residues in sialic acid-free and sialic acid-containing samples. Hydrolysis of α_1-acid glycoprotein with acid in the range pH 1–7 showed [227] that 2 mol. of sialic acid/mol. of protein were relatively strongly bound. It was shown that sialyl residues in α_1-acid glycoprotein did not influence significantly the conformational transitions of its peptide moiety and it was concluded [228] that the Cotton effect of glycoproteins was essentially due to the conformation of their polypeptide moieties.

[222] T. S. A. Samy, *Arch. Biochem. Biophys.*, 1967, **121**, 703.
[223] D. L. Grant, W. G. Martin, and P. A. Anastassihdis, *J. Biol. Chem.*, 1967, **242**, 3912.
[224] R. J. Winzler, E. D. Harris, D. J. Pekas, C. A. Johnson, and P. Weber, *Biochemistry*, 1967, **6**, 2195.
[225] T. Sato, Z. Yosizawa, M. Masubuchi, and F. Yamauchi, *Carbohydrate Res.*, 1967, **5**, 387.
[226] R. C. Hughes and R. W. Jeanloz, *Biochemistry*, 1966, **5**, 253.
[227] K. Schmid, A. Polis, K. Hunziker, R. Fricke, and M. Yayoshi, *Biochem. J.*, 1967, **104**, 361.
[228] K. Yamagami and K. Schmid, *J. Biol Chem.*, 1967, **242**, 4176.



(31)

The terminal sequence for some of the oligosaccharide moieties of ovine α_1-glycoprotein has been postulated [229] as sialylgalactosyl-2-acetamido-2-deoxyglucose. The native glycoprotein inhibited the haemagglutinating properties of Newcastle disease virus, and removal of 74–77% of the sialyl residues gave a product which was 10% less active as an inhibitor.

One glycopeptide, *M* 950, isolated from homogeneous Ba-α_2-glycoprotein of pooled human serum by sequential removal of sialic acid, proteolytic digestion, and purification, contained a disaccharide of 2-amino-2-deoxyglucose and galactose.[230] In the original glycoprotein, sialic acid was probably linked to galactose and the molecule contained a glycopeptide bond in which aspartic acid was linked *N*-glycosidically to 2-amino-2-deoxyglucose. Glycopeptides have also been isolated from human haptoglobins, the genetically determined serum α_2-glycoproteins which bind haemoglobin.[231] Two types of glycopeptide, *M* 2000–3000, with essentially similar carbohydrate compositions but different amino-acid compositions, were obtained from haptoglobin type 2-1. The carbohydrate

[229] B. J. Campbell, A. L. Schneider, D. N. Howe, and D. P. Durand, *Biochim. Biophys. Acta*, 1967, **148**, 137.
[230] K. Ishihara and K. Schmid, *Biochemistry*, 1967, **6**, 112.
[231] C. M. Gerbeck, A. Bezkorovainy, and M. E. Rafelson, jun., *Biochemistry*, 1967, **6**, 403.

moieties contained 5–6 mol. of hexose (galactose and mannose), 3–4 mol. of 2-acetamido-2-deoxyglucose, and 0–3 mol. *N*-acetylneuraminic acid. Fucose was also present in some of the glycopeptides. Residues of aspartic acid were probably involved in the glycopeptide bonds of both types of glycopeptide. The carbohydrate and amino-acid compositions of some of the glycopeptides isolated similarly from haptoglobin type 2-2 were essentially the same as the type 2-1 glycopeptides.

Blood-group Substances.—Two blood-group specific pentasaccharides have been isolated [232] from the urine of human A_1 and B secretors. The A_1 pentasaccharide contained fucose, galactose, glucose, and 2-acetamido-2-deoxygalactose (2 : 1 : 1 : 1) and the B pentasaccharide contained fucose, galactose, and glucose (2 : 2 : 1). In both oligosaccharides glucose was the reducing end-group. Oligosaccharide A_1 inhibited the A_1-anti-A_1 system, and B inhibited the B-anti-B and O-anti-H systems. The oligosaccharides may be related to blood-group active glycolipids present on the surface of red cells. A fucose-containing glycolipid isolated from human adenocarcinoma contained the structure β-D-galactopyranosyl (1 → 3, or 4)-2-acetamido-2-deoxy-D-glucose.[233] It showed moderate blood-group Le^a activity, weak H activity, and no A or B activity. Reactions with antisera to the tumour glycolipid suggested that, relative to blood-group B and H substances, blood-group A substance contained increased amounts of a hapten structurally similar to, or identical with, the carbohydrate moiety of the tumour glycolipid.

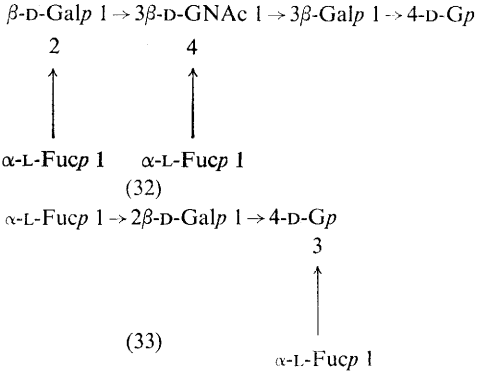
Human types MM, NN, and MN blood-group substances have been characterised [234] as glycoproteins containing *N*-acetylneuraminic acid (23 mol.), 2-acetamido-2-deoxygalactose (16 mol.), 2-acetamido-2-deoxyglucose (8 mol.), and galactose (24 mol.) based on a molecular weight for the glycoprotein of 30,000. All three substances had very similar amino-acid contents and were rich in seryl and threonyl residues which might be involved in glycopeptide linkages, and all three glycoproteins were equally susceptible to neuraminidase. The glycopeptide linkages in the native glycoproteins were more resistant to cleavage by alkali than in the sialic acid-free material. Analytical studies suggested [235] that the carbohydrate moieties of M-, N-, and MN-active sialoglycopeptides released from human erythrocytes by treatment with pronase had many structural similarities.

C-Reactive protein in acute-phase serum was shown [236, 237] to be in equilibrium with serum glycosaminoglycans which had many immunologic and serologic characteristics specifically associated with blood-group H, Le^a, and Le^b substances.

[232] A. Lundblad, *Biochim. Biophys. Acta*, 1967, **148**, 151.
[233] S.-I. Hakomori, J. Koscielak, K. J. Bloch, and R. W. Jeanloz, *J. Immunol.*, 1967, **98**, 31.
[234] R. H. Kathan and A. Adamany, *J. Biol. Chem.*, 1967, **242**, 1716.
[235] S. Ohkuma and T. Shinohara, *Biochim. Biophys. Acta*, 1967, **147**, 169.
[236] Y. Hokama, M. K. Coleman, and R. F. Riley, *J. Immunol.*, 1967, **98**, 521.
[237] Y. Hokama, M. K. Coleman, and R. F. Riley, *J. Immunol.*, 1967, **98**, 529.

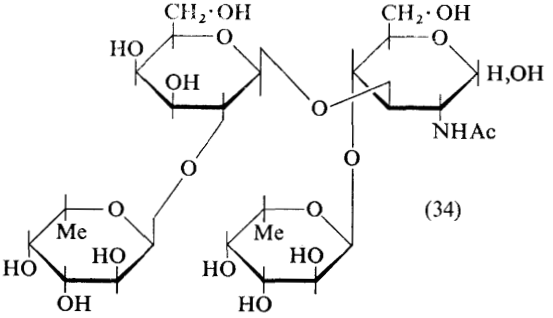
A protein which bound specifically L-fucose and agglutinated group O human red blood cells was isolated [238] from seeds of *Lotus tetragonolobus* by specific precipitation with 1,3,5-tri-[-*p*-(α-L-fucosyloxy)phenylazo]-2,4,6-trihydroxybenzene. The latter compound was prepared from *p*-aminophenyl α-L-fucopyranoside by diazotisation and coupling to phloroglucinol.

Alkaline degradation studies have yielded further information on the fine structure of the blood-group substances. Inhibition in the Le^b system by the oligosaccharides lacto-*N*-difucohexaose I (32) and lactodifucotetraose (33) have indicated [239] the nature of the Le^b determinant. Direct

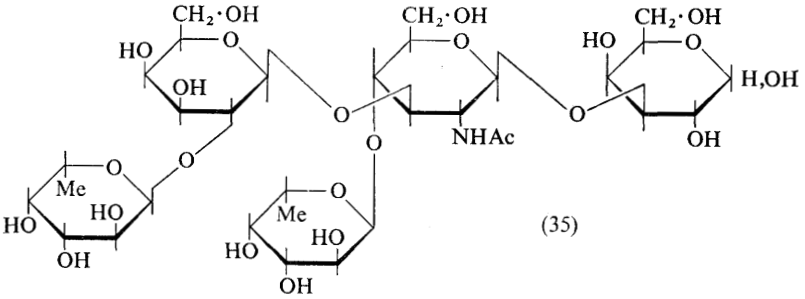


chemical evidence for the nature of the determinant was provided [240] by the isolation and characterisation from HLe^b substance of two Le^b-active oligosaccharides. HLe^b substance (fucose 13·4%, galactose 22·6%, 2-acetamido-2-deoxyhexose 25·7%, amino-acids 25%; molar ratio 2-acetamido-2-deoxygalactose to 2-acetamido-2-deoxyglucose 1 : 2·9) from ovarian cyst fluid was treated in solution with a soluble, nondialysable poly(vinyl benzene triethyl)ammonium hydroxide resin (carbonate form) at pH 8·5–8·8 for 90 days with intermittent heating to 100° and continuous dialysis of the reaction mixture. Gel chromatography of the diffusate showed two components which were subsequently purified and characterised as a tetrasaccharide and a pentasaccharide. Structural studies involving analysis, colorimetric reactions, partial hydrolysis, alkaline-degradation, methylation, and periodate oxidation indicated that the tetrasaccharide (34) was *O*-α-L-fucosyl-(1 → 2)-*O*-β-D-galactosyl-(1 → 3)-[-*O*-α-L-fucosyl-(1 → 4)-]-2-acetamido-2-deoxy-D-glucose and that the pentasaccharide (35) was *O*-α-L-fucosyl-(1 → 2)-*O*-β-D-galactosyl-(1 → 3)-[*O*-α-L-fucosyl-(1 → 4)-]-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-(1 → 3)-D-galactose.

[238] J. Yariv, A. J. Kalb, and E. Katchalski, *Nature*, 1967, **215**, 890.
[239] W. M. Watkins and W. T. J. Morgan, *Vox Sanguinis*, 1962, **7**, 129.
[240] A. M. S. Marr, A. S. R. Donald, W. M. Watkins, and W. T. J. Morgan, *Nature*, 1967, **215**, 1345.



(34)



(35)

The Le^b tetrasaccharide and pentasaccharide both inhibited in the Le^b system at low concentrations of inhibitor, but not in the Le^a or H systems. Treatment of the Le^b active pentasaccharide with an enzyme from *Trichomonas foetus*, which catalysed the release of L-fucosyl residues linked (1 → 2) to D-galactose, gave a tetrasaccharide with the structure of the Le^a determinant. This was endorsed by serological studies.

Treatment of blood-group A, B, and H substances with sodium hydroxide–sodium borohydride has been shown to give oligosaccharides, many of which were terminated at their reduced ends by a hex-3-en-1,2,5,6-tetrol (*R*) residue.[241-243] This residue arose from a D-galactopyranosyl branch point present in the intact blood-group substances by alkaline degradation of the main chains down to the branch point, and subsequent alkaline elimination of oligosaccharide side-chains.

Three oligosaccharides, each containing one fucosyl residue, and three oligosaccharides, each containing two fucosyl residues, were obtained from A, B, and H substances by treatment with sodium hydroxide–sodium

[241] K. O. Lloyd and E. A. Kabat, *Biochem. Biophys. Res. Comm.*, 1964, **16**, 385.
[242] K. O. Lloyd, E. A. Kabat, E. J. Layug, and F. Gruezo, *Biochemistry*, 1966, **5**, 1489.
[243] K. O. Lloyd and E. A. Kabat, *Carbohydrate Res.*, 1967, **4**, 165.

borohydride.[244] H-Active oligosaccharide was characterised as α-L-fucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-*R*. The corresponding A and B oligosaccharides had 2-acetamido-2-deoxy-D-galactopyranosyl and D-galactosyl residues, respectively, linked α(1 → 3) to the galactosyl residue of this structure. In the other oligosaccharides a second fucosyl residue was linked to C-3 of the 2-acetamido-2-deoxy-D-glucosyl residue of the corresponding oligosaccharide which contained only one fucosyl residue. The Cotton effect trough at 220 mμ for the oligosaccharides was attributed [244] to the 2-acetamido-group of the 2-acetamido-2-deoxyhexosyl residue. The same chromophore was responsible for the strong ellipticity bands in the circular dichroism spectra of the oligosaccharides. The oligosaccharides that contained two fucosyl residues showed low activities, as compared with those of the corresponding oligosaccharides containing one fucosyl residue, in inhibition of haemagglutination and precipitation, and it was suggested that the presence of a second fucosyl group either prevented access of antibody to the combining site or caused a conformational change such that the determinant reacted less effectively with the antibody combining site.

Subsequent studies [243] established the stereochemistry of the hex-3-ene-tetrol residue (*R*). *cis*-Hydroxylation of authentic samples of the *trans-erythro-* and *trans*-D-*threo*-forms of the alditol gave DL-glucitol from the former, and D-mannitol and D-iditol from the latter. Subsequent *cis*-hydroxylation and hydrolysis of three of the oligosaccharides which contained unsaturated alditol residues gave mixtures of these three hexitols, identified by g.l.c. of their hexacetates, thus showing that each oligosaccharide was a mixture of two oligosaccharides, one terminated by a *trans*-hex-3-ene-D-*threo*-tetrol residue and the other by a *trans*-hex-3-ene-*erythro*-tetrol residue. The formation of both the D-*threo*- and *erythro*-isomers of the hexene-tetrol probably arose from borohydride reduction of a 2-oxo intermediate of D-galactose.

Sequential hydroxylation and periodate oxidation of the disaccharides 2-acetamido-2-deoxy-β-D-glucosyl-*R* established [243] that the 2-acetamido-2-deoxyhexosyl residue was linked to C-6 of the hexen-tetrol, and, therefore, to the C-6 position of a D-galactosyl residue in the intact blood-group substance.

The presence or absence of reducing agents in such alkaline degradation reactions was important in determining the positions in the carbohydrate chains at which the so-called peeling reaction stopped.[243] In the unsaturated oligosaccharides cited above the oligosaccharide chain was relatively alkali-stable by virtue of its linkage to the C-6-hydroxy-group of a galactosyl group in the intact polysaccharide chain. Consequently, the sugar residue was reduced to the corresponding alditol before elimination of the C-6 substituent could occur to any appreciable extent. In the presence of

[244] K. O. Lloyd, S. Beychok, and E. A. Kabat, *Biochemistry*, 1967, **6**, 1448.

triethylamine, however, the C-6 substituent was eliminated, liberating oligosaccharides terminated by 2-acetamido-2-deoxy-D-glucosyl residues. A reducing oligosaccharide, tentatively identified as α-L-fucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose has also been isolated after treatment of blood-group (A + H) substance with sodium hydroxide alone.

From the results of acid and alkaline degradation studies of blood-group A substance it has been suggested [245] that the molecule consists of a central peptide chain to which are attached oligo- and/or poly-saccharide chains by *O*-glycosidic bonds involving residues of serine and threonine.

Miscellaneous Glycoproteins

Purified kallikrein from pig pancreas has been characterised [246] as a glycoprotein containing 2-amino-2-deoxyglucose (3·0–3·3%), galactose (0·7%), mannose (2%), fucose (1%), and sialic acid (0·8%). Some of the kallikreins isolated contained no sialic acid, but the presence or absence of that sugar residue was not related to biological activity. A molecular weight of 33,400–33,800 was proposed for sialic acid-free kallikrein which contained 283 amino-acid residues and 19–21 sugars residues per molecule.

Sequential treatment of bovine renal glomerular basement membrane with collagenase and pronase produced two distinct types of glycopeptides which contained very few amino-acid residues.[247] Structural studies showed[248] that one type had the structure 2-*O*-(α-D-glucopyranosyl)-*O*-(β-D-galactopyranosyl)hydroxylysine. In the other type the carbohydrate unit, which was probably linked to asparagine, was a heteropolysaccharide of average molecular weight 3500 containing galactose, mannose, 2-amino-2-deoxyhexoses, sialic acid, and fucose.[247]

Glycoproteins have been isolated [249] from the external mucous secretions of fish. The major fraction isolated from the mucin of plaice (*Pleuronectes platessa*) by gel filtration and ion-exchange methods contained fucose (5·8%), galactose (4·7%), glucose (1·9%), mannose (1·4%), 2-amino-2-deoxygalactose (8·4%), 2-amino-2-deoxyglucose (2·9%), and *N*-acetylneuraminic acid (< 1%). The principal amino-acid components were threonine (9·8%), serine (5·4%), glutamic acid (5·2%), and aspartic acid (4·3%). Two minor glycoprotein fractions had similar sugar, but different amino-acid, compositions and contained ester sulphate. Immunochemical and other evidence indicated that whole-plaice serum contained the major glycoprotein component of the mucin.

Sulphate bound to glycoprotein has also been found in epithelial mucus and in gastric secretion and extracts from the mucous glands of the

[245] N. K. Kochetkov, V. A. Dervitskaya, and S. G. Kara-Murza, *Carbohydrate Res.*, 1967, **3**, 403.
[246] H. Fritz, I. Eckert, and E. Werle, *Z. Physiol. Chem.*, 1967, **348**, 1120.
[247] R. G. Spiro, *J. Biol. Chem.*, 1967, **242**, 1923.
[248] R. G. Spiro, *J. Biol. Chem.*, 1967, **242**, 4813.
[249] T. C. Fletcher and P. T. Grant, *Biochem. J.*, 1968, **106**, 12P.

gastric body mucosa and antrum.[250] Analytical studies indicated that macromolecular carbohydrate fractions of gastric secretion were polydisperse with respect to sulphate and *N*-acetylneuraminic acid, but contained no major structural differences in backbone structures which comprised the sugar components fucose, galactose, mannose, glucose, 2-amino-2-deoxygalactose, and 2-amino-2-deoxyglucose. The two aminosugars and galactose formed 70–80% of the carbohydrate content of the secretion, and residues of fucose and *N*-acetylneuraminic acid occupied terminal positions. The carbohydrate components of extracts from the mucous glands of the body mucosa and antrum were not different from those of gastric secretion.

Further studies have confirmed that purified mammalian gonadotrophins such as follicle-stimulating hormone (FSH) and lutenising hormone (LH) are glycoproteins.[251] The carbohydrate moiety of such molecules from several species has been shown to contain varying proportions of D-galactose, mannose, L-fucose, 2-acetamido-2-deoxyglucose, and *N*-acetylneuraminic acid.[252] The different carbohydrate compositions that have been reported for hormones from different species probably reflect the known species differences, but significant analytical variations have been reported by different laboratories for, *e.g.* human FSH. Thus, the total carbohydrate content of this molecule has been reported as 8%[253] and 40%.[254] *N*-Acetylneuraminic acid was removed from human FSH with complete loss of biological activity[251, 255] whereas either, removal, or, oxidation with D-galactose oxidase, of terminal, nonreducing residues of D-galactose did not affect biological activity.[255] *N*-Acetylneuraminic acid has been shown to augment the hormonal activity of human FSH.[256] The activity of ovine LH was retained after treatment with neuraminidase.[257] The molecular weight of human FSH in a monomeric form has been reported as *ca.* 17,000.[258] The molecule formed a dimer and tetramer with decrease of salt concentration.

[250] J. Schrager and M. D. Oates, *Biochem. J.*, 1968, **106**, 523.
[251] W. R. Butt, 'The Chemistry of the Gonadotrophins', C. C. Thomas, Springfield, Illinois, 1967.
[252] W. R. Butt, *Ann. Reports*, 1967, **69**, in the press.
[253] H. Papkoff, L.-J. Mahlmann, and C. H. Li, *Biochemistry*, 1967, **6**, 3976.
[254] P. Roos, Inaugural dissertation for Ph.D. degree, University of Uppsala, 1967.
[255] W. R. Butt, J. F. Jenkins, and P. J. Somers, *J. Endocrinol.*, 1967, **38**, xi.
[256] S. M. Amir, S. A. Barker, W. R. Butt, and A. C. Crooke, *J. Endocrinol.*, 1966, **35**, 425; *Nature*, 1966, **211**, 975.
[257] H. Papkoff, D. Gospodarowicz, A. Candiotti, and C. H. Li, *Arch. Biochem. Biophys.*, 1965, **111**, 431.
[258] C. J. Gray, *Nature*, 1967, **216**, 1112.

6
Carbohydrate Sulphates

Since this topic was reviewed by Rees,[259] several new sulphated polysaccharides have been isolated from natural sources. Structural studies have focused on the nature of the covalent linkage between individual sulphated glycosaminoglycans and the protein molecules with which they are associated in nature, and on the more complicated molecular architecture of the protein–polysaccharide complexes which may be isolated from animal tissues by relatively mild methods. Improved methods for the separation and analysis of sulphated polysaccharides have permitted comparative studies of these molecules in certain pathological conditions.

Polysaccharide Sulphates from Animal Tissues

The nomenclature of individual compounds as recommended by Jeanloz [260] will be used here.

Several methods have been reported for the isolation and separation of acidic glycosaminoglycans on a microscale. Differential solubilisation of their cetyl pyridinium salts from cellulose columns [261] gave reproducible separations of the acidic glycosaminoglycans of ground substance [262] and was used to study chemical changes in the glycosaminoglycan sulphates in histological sections of horse nasal septum cartilage.[263] Chemical analyses of 0·2-mm. thick sections of mature human cartilage substantiated [264] earlier histological evidence [265] for the locations of chondroitin sulphate and keratan sulphate that was based on staining reactions with Alcian Blue dye at different salt concentrations. Polysaccharide–protein complexes isolated from human rib cartilage showed age-dependent changes in chemical composition and macromolecular properties.[266]

The increased solubility of the cetyl pyridinium salts of glycosaminoglycan sulphates following depolymerisation with hyaluronidase has been

[259] D. A. Rees, *Ann. Reports*, 1965, **62**, 469.
[260] R. W. Jeanloz, *Arthritis and Rheumatism*, 1960, **3**, 233.
[261] C. A. Antonopoulos, S. Gardell, J. A. Szirmai, and E. R. de Tyssonsk, *Biochim. Biophys. Acta*, 1964, **83**, 1.
[262] J. Svejcar and W. van B. Robertson, *Analyt. Biochem.*, 1967, **18**, 333.
[263] J. A. Szirmai, E. van Boven-de Tyssonsk, and S. Gardell, *Biochim. Biophys. Acta*, 1967, **136**, 331.
[264] R. A. Stockwell and J. E. Scott, *Nature*, 1967, **215**, 1376.
[265] J. E. Scott and J. Dorling, *Histochemie*, 1965, **5**, 221.
[266] W. Kroz and E. Buddecke, *Z. physiol. Chem.*, 1967, **348**, 665.

exploited [267] in a micro-method for the determination of hyaluronic acid, heparan sulphate, chondroitin sulphates, and dermatan sulphate in mixtures. The 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose contents of the small molecular weight fraction represented the amounts of hyaluronic acid and chondroitin sulphates, respectively, in the original mixture, whereas in the undepolymerised fraction those two sugars represented the amounts of heparan sulphate and dermatan sulphate, respectively.[268] Other methods reported for the separation of acidic glycosaminoglycans included thin-layer chromatography on silica gel,[269] chromatography on paper [270] and on ECTEOLA-cellulose,[271] electrophoresis on gels of polyacrylamide [272] or agarose,[273] and electrophoresis on cellulose polyacetate following preliminary purification on ECTEOLA-cellulose.[274] Dermatan sulphate was resolved from chondroitin 4- and 6-sulphates by paper electrophoresis in zinc sulphate or zinc acetate buffers.[275] Chondroitin 4-sulphate was detected in papain digests of human leucocytes and platelets.[276]

Comparative chemical studies of bovine cartilage and bone showed [277] that the content of chondroitin sulphates in epiphyseal plate cartilage (33·9%) was greater than that of articular cartilage (25·3%), primary and secondary spongiosa of metaphyses (1·9% and 1·1%, respectively) or compact cortical bone (0·8%). The keratan sulphate contents of these materials were 4·36, 3·69, 3·29, 0·70, and 0·86%, respectively. The keratan sulphate content of bovine invertebral tissue was shown to increase during embryonic development but was less than half that present in the same tissue from mature animals.[278] Aqueous extracts of bovine corneal stroma contained glucosamino- and galactosamino-glycans covalently linked to protein, in the former by alkali-stable bonds, in the latter by alkali-labile bonds.[279] The total content of sulphated glycosaminoglycans in bovine whole brain was only 0·03% and comprised chondroitin 4- and 6-sulphates and hyaluronic acid of molecular weight *ca.* 14,000.[280] A large part of the brain glycosaminoglycans was not of connective tissue origin.

Studies of the influence of cortisol on the synthesis of sulphated glycosaminoglycans and collagen in chick embryos have suggested,[281] either, that

[267] S. Thunell, *Arkiv Kemi*, 1967, **27**, 33.
[268] S. Thunell, *Arkiv Kemi*, 1967, **27**, 45.
[269] G. Marzullo and J. W. Lash, *Analyt. Biochem.*, 1967, **18**, 575.
[270] T. A. Good, *Analyt. Biochem.*, 1967, **19**, 109.
[271] D. A. Lowther, B. P. Toole, and F. A. Meyer, *Arch. Biochem. Biophys.*, 1967, **118**, 1; C. A. Antonopoulos, L.-Å. Fransson, D. Heinegård, and S. Gardell, *Biochim. Biophys. Acta*, 1967, **148**, 158.
[272] O. M. Rennert, *Nature*, 1967, **213**, 1133.
[273] A. A. Horner, *Canad. J. Biochem.*, 1967, **45**, 1009.
[274] V. Stefanovich and I. Gore, *J. Chromatog.*, 1967, **31**, 473.
[275] F. Haruki and J. E. Kirk, *Biochim. Biophys. Acta*, 1967, **136**, 391.
[276] I. Olsson and S. Gardell, *Biochim. Biophys. Acta*, 1967, **141**, 348.
[277] R. D. Campo and C. D. Tourtellotte, *Biochim. Biophys. Acta*, 1967, **141**, 614.
[278] J. A. Cifonelli, A. Saunders, and J. I. Gross, *Carbohydrate Res.*, 1967, **3**, 478.
[279] H. L. Kern and D. Brassil, *Arch. Biochem. Biophys.*, 1967, **118**, 115.
[280] R. U. Margolis, *Biochim. Biophys. Acta*, 1967, **141**, 91.
[281] P. S. Ebert and D. J. Prockop, *Biochim. Biophys. Acta*, 1967, **136**, 45.

cortisol has separate but concomitant effects on protein and glycosaminoglycan synthesis, or, that the inhibition of the synthesis of glycosaminoglycans is secondary to the general inhibition of protein synthesis. The low content of chondroitin 4-sulphate in cartilage from nanomelic chick embryos probably resulted from deficient biosynthesis.[282] The metabolism of chondroitin sulphates in epiphyseal cartilage of rachitic chicks was increased [283] by vitamin D_3, and the chondroitin sulphate content of rat retina and aorta increased significantly in alloxan diabetes.[284]

Hydroxylysine-containing compounds have previously been reported [285] in the urine of patients recovering from severe burns and glycosides in which C-1 of glucosylgalactose or of galactose was linked to the δ-hydroxy-group of hydroxylysine have now been isolated [200b] from normal human urine. Identical compounds were present in alkaline hydrolysates of guinea-pig collagen. The predominant acidic glycosaminoglycans found in normal human urine were chondroitin sulphates, of varying degrees of sulphation, of which chondroitin 4- and 6-sulphates each comprised about 33% of the total glycosaminoglycans.[286] A nonsulphated chondroitin and heparan monosulphate constituted 25 and 8%, respectively, of the total, and hyaluronic acid, dermatan sulphate, and a highly sulphated keratan sulphate were excreted in trace amounts. Monosulphated chondroitin sulphate was excreted as fragments of small molecular size, some of which were covalently bound to a peptide which contained large amounts of serine, glycine, and glutamic acid.

It has been suggested [287] that acidic glycosaminoglycans in the mammalian kidney play an important role in the process of concentrating urine.

Teller suggested [288] that the urinary excretion pattern of individual acidic glycosaminoglycans is pathognomic of certain hereditary bone diseases. Normal glycosaminoglycan patterns were reported [289] in the urine of three children with nail-patella syndrome and one with Farber's disease. Moderately increased levels of sulphated and nonsulphated glycosaminoglycans were excreted in the urine of patients with diffuse scleroderma, systemic lupus erythrematosus, and rheumatoid arthritis and extremely high levels were found in the urine of patients with gargoylism (Hurler's syndrome). This has been attributed [290, 291] to an over-production of dermatan sulphate and heparan sulphate. Heparan sulphate isolated from

[282] M. B. Mathews, *Nature*, 1967, **213**, 1255.
[283] J. D. Cipera, *Canad. J. Biochem.*, 1967, **45**, 729.
[284] R. A. Patterson and H. Heath, *Biochim. Biophys. Acta*, 1967, **148**, 207.
[285] F. L. Estes and T. Golaszewski, *Fed. Proc.*, 1965, **24**, 606.
[286] D. P. Varadi, J. A. Cifonelli, and A. Dorfman, *Biochim. Biophys. Acta*, 1967, **141**, 103.
[287] G. G. Pinter, *Experientia*, 1967, **23**, 100.
[288] W. M. Teller, *Nature*, 1967, **213**, 1132.
[289] E. Wessler, *Clinica Chim. Acta*, 1967, **16**, 235.
[290] K. Meyer, M. M. Grumbach, A. Linker, and P. Hoffman, *Proc. Soc. Exptl. Biol. Med.*, 1958, **97**, 275.
[291] K. Meyer, P. Hoffman, A. Linker, M. M. Grumbach, and P. Sampson, *Proc. Soc. Exptl. Biol. Med.*, 1959, **102**, 587.

the livers and urine of patients with Hurler's syndrome was shown [292] to be heterogeneous, and chemical analyses of the fragments suggested that they might represent segments of a single parent molecule similar in structure to heparan sulphate from normal human aorta which was characterised as a polysaccharide, M_W24,000–29,000. The presence in the normal heparan sulphate of serine, D-xylose, and D-galactose in the molar proportions 1 : 1 : 2 suggested [292] that the glycopeptide linkage region was similar to that in chondroitin 4-sulphate [293] and heparin,[294] *viz.* D-glucuronosyl-D-galactosyl-D-galactosylxylosyl serine. Treatment with nitrous acid indicated [292] that a relatively large segment of 2-acetamido-2-deoxy-D-glucosyluronic acid repeating units, with very small quantities of sulphate ester was present in the region of protein binding in heparan sulphate.

Heparan sulphate, linked to protein in part, or exclusively, by alkali-labile glycosyl-threonine linkages, was the predominant glycosaminoglycan sulphate excreted in the urine of patients with Sanfillipo syndrome.[295] The isolation of a heparan-serine compound after sequential treatment of the heparan sulphate with pepsin, trypsin, and dilute aqueous alkali suggested that serine either was an integral part of the polysaccharide or was involved in an alkali-stable *O*-glycosyl linkage.

A chondroitin sulphate–protein complex, M_W250,000, which was considered to be derived from cartilage, was present in normal ox synovial fluid.[296] Chondroitin sulphate has been demonstrated chemically in human rheumatoid synovial fluid,[297] and a component closely related immunologically to the protein-polysaccharides of cartilage was detected [298] in increased amounts in synovial fluids from patients with lupus, gout, rheumatic fever, and septic arthritis. These components were probably released from articular cartilage during acute inflammation of the joint.

Structural Studies.—*General methods.* 'Finger-printing' patterns of glycopeptides isolated from proteolytic digests of acidic glycosaminoglycans have been obtained [299] by hydrolysis to oligosaccharides with Amberlite resin IR 120 (H^+ form) at 100° for 1 hr. and analysis of the hydrolysate by two-dimensional chromatography on thin layers of cellulose. Mild, but effective, hydrolysis of sulphated glycosaminoglycans to component monosaccharides was achieved [300] by use of Dowex resin 50 (H^+ form) and 0·05 M-hydrochloric acid at 100° for 24 hr. in a sealed tube. A colorimetric method for the quantitative estimation of xylosyl residues in the presence of glucuronic

[292] J. J. Knecht, A. Cifonelli, and A. Dorfman, *J. Biol. Chem.*, 1967, **242**, 4652.
[293] L. Rodén and G. Armand, *J. Biol. Chem.*, 1966, **241**, 65.
[294] U. Lindahl, *Arkiv Kemi*, 1967, **26**, 101.
[295] D. Kaplan, *Biochim. Biophys. Acta*, 1967, **136**, 394.
[296] P. Silpananta, J. R. Dunstone, and A. G. Ogston, *Biochem. J.*, 1967, **104**, 404.
[297] P. Seppälä, A. Lehtonen, J. Kärkkainen, and V. Näntö, *Clinica Chim. Acta*, 1967, **16**, 115.
[298] J. Sandson, *Science*, 1967, **155**, 839.
[299] E. Moczar and M. Moczar, *Biochem. J.*, 1967, **104**, 17P.
[300] P. L. Jeffrey and K. G. Rienits, *Biochim. Biophys. Acta*, 1967, **141**, 179.

acid, galactose, 2-amino-2-deoxyglucose, and amino-acids has been reported.[301]

The i.r. absorption spectra of synthetic D-galactose sulphates and their derivatives showed[302] that the assignment of sulphate ester groups in carbohydrate sulphates from i.r. data alone requires some caution. Absorptions at 850, 830, and 820 cm.$^{-1}$ have previously been assigned[303] to sulphate groups at secondary axial, secondary equatorial, and primary equatorial positions, respectively, on the assumption that the sugar unit had a particular conformation, usually the *C*1 chair form of the pyranose ring. Other effects, such as intermolecular forces in the solid state, might also influence the position of maximal absorption,[304] and certain sugar sulphates, *e.g.* D-galactose 2-sulphate, might exist in some conformation other than the *C*1 pyranose ring form.[302]

Further studies of the action of glycosulphatases and chondrosulphatases have indicated the potential of these enzymes in structural studies of carbohydrate sulphates. Extracts of acetone-dried cells of *Proteus vulgaris* grown on a nutrient broth medium containing either 5% w/v D-glucose or 5% w/v glycerol were unable to desulphate or depolymerise chondroitin 4- or 6-sulphates, or dermatan sulphate, but catalysed the release of $^{35}SO_4^{2-}$ ions from 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose-6-*O*-[^{35}S]sulphate (*N*-acetyl chondrosine 6-*O*-[^{35}S]sulphate).[305] In contrast, enzyme preparations from cells grown on nutrient broth alone showed both chondroitinase and glycosulphatase activities. The chondrosulphatase, freed from chondroitinase activity by chromatography on DEAE-Sephadex, was inactive against the sulphated glycosaminoglycans and the 6-sulphates of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose, but readily liberated $^{35}SO_4^{2-}$ from the labelled disaccharide. Enzymes with glycosulphatase and chondrosulphatase activities have been partially purified[306] by ammonium sulphate fractionation of extracts of the viscera of the common limpet (*Patella vulgata*). The chondrosulphatase preparation might contain an oligosaccharide–sulphatase similar to that present in *P. vulgaris* (see also p. 262).

Dermatan Sulphate.—Although L-iduronic acid is the only hexuronic acid hitherto reported as a component of dermatan sulphates from a variety of animal tissues, D-glucuronic acid was shown to be an intergal part of the dermatan sulphate molecule isolated from pig skin.[307] Treatment of the dermatan sulphate with testicular hyaluronidase gave products with

[301] F. K. Stevenson, P. W. Kent, and D. Fisher, *Chem. and Ind.*, 1967, 703.
[302] J. R. Turvey, D. M. Bowker, and M. J. Harris, *Chem. and Ind.*, 1967, 2081.
[303] S. F. D. Orr, *Biochim. Biophys. Acta*, 1954, **14**, 173; A. G. Lloyd and K. S. Dodgson, *ibid.*, 1961, **46**, 116; A. G. Lloyd, K. S. Dodgson, R. G. Price, and F. A. Rose, *ibid.*, 1961, **46**, 108.
[304] H. Spedding, *Adv. Carbohydrate Chem.*, 1964, **19**, 23.
[305] A. G. Lloyd, A. H. Olaveson, P. A. Wooley, and C. Emberry, *Biochem. J.*, 1967, **102**, 32P.
[306] P. F. Lloyd and R. J. Fielder, *Biochem. J.*, 1967, **105**, 33P.
[307] L.-Å. Fransson and L. Rodén, *J. Biol. Chem.*, 1967, **242**, 4161.

D-glucuronosyl residues in the newly formed, nonreducing termini. Since hyaluronidase-susceptible linkages to D-glucuronic acid were present in the vicinity of the linkage to peptide, a large number of the polysaccharide fragments from the hyaluronidase digest were devoid of amino-acids. Some indication of the location of D-glucuronosyl residues in the dermatan sulphate molecule was provided by the isolation,[308] from the hyaluronidase digest, of a tetrasaccharide (36). An analogous, unsulphated tetrasaccharide was isolated by acid hydrolysis of the dermatan sulphate, thus excluding the possibility that it arose from transglyosylation.

D-GA—GalNAcSO$_4$—Id A—GalNAcSO$_4$

(36)

Heparin.—Location of the sulphate groups in glycosaminoglycans, especially in heparin, is one of the more difficult aspects of structural studies of such molecules. Chemical proof of the presence of residues of 2-amino-2-deoxy-D-glucose 6-sulphate in heparin has been obtained.[309] Oxidation of purified heparin with periodate, until exactly 1 mol. of oxidant per tetrasaccharide unit was consumed, apparently cleaved the glycol group of the unsubstituted D-glucuronosyl residue. The product of oxidation was sequentially reduced with sodium borohydride and hydrolysed with dilute acid which cleaved the acetal linkage of the oxidised group with concomitant *N*-desulphation. Subsequent deamination with nitrous acid gave four components, separable by electrophoresis in borate buffer, one (M_{gl} 0·84) of which gave a crystalline brucinium salt indistinguishable from the same derivative of authentic 2,5-anhydro-D-mannose 6-(sodium sulphate).

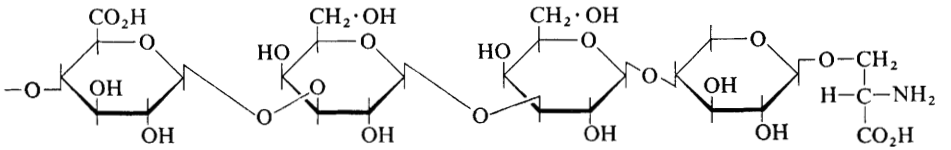
Isolation and characterisation of a disaccharide (37) and a trisaccharide (38) from acid hydrolysates (pH 3, at 100° for 42 hr.) of heparin confirmed[294] that the glycopeptide linkage (39) in heparin was identical with that in the chondroitin 4-sulphate–protein complex.[293]

β-D-G*p*GA 1→3-D-Gal

(37)

β-D-Gal*p* 1→3β-D-Gal*p* 1→4-D-Xyl

(38)



(39)

[308] L.-Å. Fransson and L. Rodén, *J. Biol. Chem.*, 1967, **242**, 4170.
[309] M. L. Wolfrom and P. Y. Wang, *Chem. Comm.*, 1967, 241.

Analyses of heparin from bovine, procine, and whale organs showed [310] that the terrestial mammals have α-heparin whereas whale has ω-heparin. The latter was less sulphated and more dextrorotatory than α-heparin. Alterations in the anticoagulant and lipolytic activities of bovine α-heparin and whale ω-heparin on mild treatment with acid have been reported.[311]

Whale ω-heparin was degraded by a crude enzyme from heparin-adapted flavobacterium to an α-oxo-acid, *N*-sulphated and *N,O*-disulphated 2-amino-2-deoxyglucose, and minor products of 2-acetamido-2-deoxy-D-glucose and oligosaccharides containing 2-acetamido-2-deoxy-D-glucose.[312] Digestion of the heparin with an eliminase separated from the crude enzyme gave several unsaturated oligosaccharides, two of which contained unsaturated uronic acid and 2-amino-2-deoxyglucose with and without sulphate. These studies suggested that ω-heparin was a complex polysaccharide in which α-heparin-like polysaccharides were attached to a polysaccharide containing 2-acetamido-2-deoxy-D-glucose.

N.m.r. studies of α- and ω-heparins and of heparan sulphate indicated [310] that all the polymers contained residues of 2-amino-2-deoxyglucose 6-sulphate and α-glycosidic linkages. O.r.d. spectra of the heparins showed weak, positive, Cotton effects at 225 mμ, whereas chondroitin sulphates gave clear, negative, Cotton effects at 220 mμ. These effects were useful for investigating the conformational alterations which heparin underwent with changes in pH or on addition of biogenic amines.[313] The Cotton effect originated in the degree of order involving the sulphamino-groups and showed, for example, that binding of histamine stabilised a given molecular order in heparin. Studies of the induced Cotton effects in Methylene Blue–heparin complexes supported the conclusion that the anionic sites of heparin in solution were in helical order.[314] In a molecular model of heparin the specific conformation was stabilised by a linear hydrogen-bond involving the nitrogen atom of the sulphamino-group as the acceptor and the hydroxy-group at C-3 of the following uronic acid as the donor.

Changes in the absorption spectra of heparin–Azure A complexes in aqueous solution have been studied [315] and the thermodynamic parameters of the interaction evaluated. The observed spectral changes may be attributed to the interactions between anionic sites of the polyanion and dye counter-ions. Light-induced paramagnetism was observed [316] in solid glycosaminoglycan–cationic dye complexes as a result of interaction between the anionic sites and the cationic dye.

[310] T. Kotoku, Z. Yosizawa, and F. Yamauchi, *Arch. Biochem. Biophys.*, 1967, **120**, 553.
[311] Z. Yosizawa, T. Kotoku, F. Yamauchi, and M. Matsuno, *Biochim. Biophys. Acta*, 1967, **141**, 358.
[312] Z. Yosizawa, *Biochim. Biophys. Acta*, 1967, **141**, 600.
[313] A. L. Stone, *Nature*, 1967, **216**, 551.
[314] A. L. Stone and H. Moss, *Biochim. Biophys. Acta*, 1967, **136**, 56.
[315] M. D. Young, G. O. Phillips, and E. A. Balazs, *Biochim. Biophys. Acta*, 1967, **141**, 374.
[316] E. A. Balazs, G. O. Phillips, and M. D. Young, *Biochim. Biophys. Acta*, 1967, **141**, 382.

Keratan Sulphate.—Methylation studies of per-acetylated bovine corneal keratan sulphate showed [317] that the main repeating unit was 2-acetamido-2-deoxy-[4-*O*-β-D-galactopyranosyl]-D-glucose, (*N*-acetyl-lactosamine), polymerised *via* a (1 → 3) linkage to D-galactose. Approximately 40% of the D-galactosyl residues and 74% of the 2-acetamido-2-deoxy-D-glucosyl residues were substituted at C-6, at least the major part by sulphate ester groups as was indicated by the isolation of galactose 6-sulphate by partial hydrolysis with acid. The polymer possibly contained branch points, and earlier reports [318] of the presence of small amounts of L-fucose in keratan sulphate were confirmed [319] by the isolation and characterisation of L-fucose, as its *N*-methyl-*N*-phenyl hydrazone and as L-fucitol, from hydrolysates of human skeletal keratosulphate.

Keratan sulphate from old human rib cartilage was polydisperse on Bio-Gel resins. Treatment of a higher molecular weight fraction with alkali gave a polydisperse product resulting from the cleavage of bonds involving 2-acetamido-2-deoxygalactose as well as serine and threonine.[319] Further alkaline degradation studies in the presence and absence of borohydride showed that the predominant *O*-glycosyl group linked to the hydroxyamino-acids was 2-acetamido-2-deoxygalactose. The decrease in seryl or threonyl groups on treatment with alkali was paralleled by the production of a direct Ehrlich's chromogen which appeared to be still attached to a high molecular weight product. This indicated that 2-acetamido-2-deoxy-galactosyl residues were linked glycosidically in the original keratan sulphate to the hydroxy-groups of the hydroxyamino-acids, and had an alkali-labile substituent at C-3 and an alkali-stable substituent at C-6. These conclusions were necessarily based on statistical data since the original material was isolated from pooled cartilage, but keratan sulphate isolated from one individual showed similar behaviour on treatment with alkali and alkaline borohydride.

Chondroitin Sulphates.—Chondroitin 4-sulphate–protein complexes extracted with neutral calcium chloride solution from pig laryngeal cartilage and purified by precipitation with 9-aminoacridine were separated into two fractions on electrophoresis at pH 7·2 on glass fibre.[320] The fractions had different antigenic determinants and differed also in content of protein, hexose, and 2-amino-2-deoxyglucose. The fraction of higher mobility had molecular weight 230,000, by sedimentation, and contained 2% of protein and only traces of 2-amino-2-deoxyglucose. Over half the amino-acid residues were serine and glycine, the former probably linked to the chondroitin 4-sulphate chains. The fraction of lower electrophoretic mobility resembled the protein-polysaccharide light fraction (PPL) in chemical analysis, and probably contained some keratan sulphate.

[317] V. P. Bhavanandan and K. Meyer, *J. Biol. Chem.*, 1967, **242**, 4352.
[318] S. Gardell, *Acta Chem. Scand.*, 1957, **11**, 668.
[319] B. A. Bray, R. Lieberman, and K. Meyer, *J. Biol. Chem.*, 1967, **242**, 3373.
[320] H. Muir and S. Jacobs, *Biochem. J.*, 1967, **103**, 367.

Purified chondroitin 4-sulphate–protein complexes from bovine nasal cartilage contained 12–18% of protein free from hydroxyproline.[321] The molecular weight, determined by light scattering, was approximately 3×10^6 and the rod-shaped molecules, length 3000 Å and radius of gyration of 850–1350 Å, showed a strong tendency to form molecular aggregates in electrolyte solutions.

Three homogeneous chondroitin 4-sulphate–peptides, isolated and purified by chromatography on ECTEOLA-cellulose, of a proteolytic digest of cornea contained aspartic acid, glutamic acid, serine, alanine, and glycine as the predominant amino-acids, and the neutral sugars, xylose and galactose, but differed in their sulphate contents.[322, 323] Threonyl and seryl residues in all three glycopeptides were partly destroyed by β-elimination after treatment with alkali, and treatment with enzymes from *Proteus vulgaris* converted the glycopeptides at equal rates into unsaturated uronides.

An enzyme, designated chondroitin sulphatase (chondroitin 4-sulphate sulphohydrolase EC 3.1.6.4), which catalysed the release of inorganic sulphate from chondroitin 4-sulphate, has been purified[324] from bovine aorta by precipitation with ammonium sulphate. The enzyme showed no detectable catalytic activity with chondroitin 6-sulphate, dermatan sulphate, or keratan sulphate as substrates. It was free from hyaluronidase activity and the rate of release of sulphate ions from chondroitin 4-sulphate was not increased by the simultaneous action of the sulphatase and hyaluronidase.

Molecular Architecture of Glycosaminoglycan–proteins in Animal Tissues.—Considerable evidence has been presented for the existence in animal tissues of hybrid molecules containing two or more different glycosaminoglycans associated with the same protein. The isolation from cartilage protein–polysaccharide of two essentially similar glycoproteins containing, in addition to peptide, uronic acid (1·0–1·5%), 2-amino-2-deoxyhexose (2·4–2·7%), 2-amino-2-deoxygalactose (0·9–1·3%), and hexose (7·5–8·4%) supported the concept that 'bridging' proteins were involved in the formation of the three-dimensional structure of the complex.[325] Glucose and galactose were tentatively identified in hydrolysates of the glycoprotein.

Extraction of bovine cartilage at 37° without destruction of the collagenous structure gave protein–polysaccharides which contained variable ratios of chondroitin sulphate chain to protein, whereas extraction by more vigorous methods gave products from which single chains of chondroitin sulphate and multichain protein–polysaccharides could be distinguished.[326]

[321] E. Buddecke, W. Kröz, and W. Tittor, *Z. physiol. Chem.*, 1967, **348**, 651.
[322] H. Greiling, H. W. Stuhlsatz, and L. Plagemann, *Z. physiol. Chem.*, 1967, **348**, 121.
[323] H. Greiling, H. W. Stuhlsatz, and R. Kisters, *Z. physiol. Chem.*, 1967, **348**, 970.
[324] E. Held and E. Buddecke, *Z. physiol. Chem.*, 1967, **348**, 1047.
[325] J. R. Dunstone and M. D. Franek, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 39.
[326] P. Hoffman, T. A. Mashburn, jun., K. Meyer, and B. A. Bray, *J. Biol. Chem.*, 1967, **242**, 3799.

Fractions richer in chondroitin sulphate chains showed a corresponding increase in alkali-sensitive serine residues which were probably not sequentially located in a core protein. Three glycosaminoglycan fractions, prepared by ultracentrifugal analysis of protein–polysaccharide from bovine nasal cartilage, each contained chondroitin sulphate and keratan sulphate in the polysaccharide moiety.[327]

A purified protein–polysaccharide complex extracted with water from human nucleus pulposus contained 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose and could not be fractionated into components containing only one of these sugars.[328] It was suggested that the two main polysaccharides of human nucleus pulposus were linked to the same protein core and similar conclusions were made[329] for the gross structure of fractions isolated from aqueous extracts of bovine nucleus pulposus. One fraction, homogeneous by ultracentrifugation and zone electrophoresis, had a weight average molecular weight of 171,000. The fractions contained different proportions of protein, chondroitin 6-sulphate, and keratan sulphate.

Chondroitin sulphate and keratan sulphate were shown to be part of the same protein–polysaccharide complex in bovine nasal septum.[330] Single chains of chondroitin sulphate, with a characteristic peptide attached, were released by papain digestion of the protein–polysaccharide. A larger molecular weight fraction of the digest contained the keratan sulphate, which retained a peptide with a different distribution of amino-acids, and some chondroitin sulphate chains that could be released by alkaline treatment with concomitant loss of serinyl and threonyl residues involved in glycosidic linkages to the chondroitin sulphate molecules. An antigenically distinct hybrid protein–polysaccharide of keratan sulphate and chondroitin sulphate, in which residues of both serine and threonine were involved in glycosidic linkages to the keratan sulphate, has been demonstrated[331] in pig laryngeal cartilage. Preliminary investigations of glycosaminoglycans from human aortic wall have suggested[45] the existence of a hybrid molecule containing chondroitin 4-sulphate, chondroitin 6-sulphate, and dermatan sulphate.

Protein–polysaccharide complexes prepared in three different ways from bovine nasal septa showed very similar chemical compositions (see Table 2) and no evidence of gross inhomogeneity.[332] Very little is known about the PPH fraction; it contained easily hydrolysable glucose and 60% of its high protein content was attributable to collagen. PPL contained chondroitin, keratan sulphate, and glycoprotein. Cartilage from cattle of one age group was used to avoid the possibility of age variations in the ratio of chondroitin

[327] M. D. Franek and J. R. Dunstone, *J. Biol. Chem.*, 1967, **242**, 3460.
[328] D. Heinegård and S. Gardell, *Biochim. Biophys. Acta*, 1967, **148**, 164.
[329] L. Rosenberg, M. Schubert, and J. Sandson, *J. Biol. Chem.*, 1967, **242**, 4691.
[330] P. Hoffman, T. A. Mashburn, jun., and K. Meyer, *J. Biol. Chem.*, 1967, **242**, 3805.
[331] C. P. Tsiganos and H. M. Muir, *Biochem. J.*, 1967, **104**, 26C.
[332] M. Luscombe and C. F. Phelps, *Biochem. J.*, 1967, **102**, 110.

4-sulphate to keratan sulphate and chondroitin 6-sulphate, and sedimentation, viscosity, and light-scattering measurements of the PPL fraction suggested a molecule of molecular weight $3{\cdot}2 \times 10^6$—$5{\cdot}8 \times 10^6$ with a spherical or slightly ellipsoidal domain, and an average radius of gyration of 1390 Å. The particle was highly solvated and charged with sulphate groups on the

Table 2

	Composition (% of dry weight)			
	PPL[a]	PPH[b]	UFR[c]	CPP[d]
Hexuronic acid	25·2	9·7	24·6	24·0
2-Amino-2-deoxyhexose	26·7	10·7	26·1	24·5
Molar ratio 2-amino-2-deoxyhexose: hexuronic acid	1·14	—	1·16	1·10
Protein (biuret)	20·3	61·6	16·8	17·9
Galactose (anthrone)	4·6	7·2	4·3	5·0
Sulphur	4·5	2·15	4·4	—
Sialic acid	1·11	0·74	0·95	0·97
Nitrogen	5·2	—	4·4	—

[a] Protein–polysaccharide light fraction resolved by ultracentrifugation.
[b] Protein–polysaccharide heavy fraction resolved by ultracentrifugation.
[c] Ultrafilter residue.
[d] Cetylpyridinium-precipitated complex.

outside. Luscombe and Phelps suggested [332] that the macromolecule was probably composed of aggregates of protein cores with randomly coiled chains of chondroitin sulphate which comprised a three-dimensional network in which keratan sulphate and glycoprotein molecules were sterically trapped. Keratan sulphate was only released by enzymic degradation. Subsequent studies [333] of the action of degradative enzymes on PPL shed further light on the structure of the complex. The weight average molecular weight of the trypsin-digested complex was $2{\cdot}1 \times 10^4$, a value consistent with those reported by other workers. Treatment of PPL with papain or with alkali at low temperature gave fragments of similar molecular weights, although the latter preparation contained no protein. These results supported earlier evidence that papain acted to produce single polysaccharide chains whereas trypsin produced fragments with two polysaccharide chains. Viscosity measurements supported this hypothesis. The weight average molecular weight of the protein core remaining after digestion with hyaluronidase was 12×10^4—14×10^4 in agreement with earlier values.

Luscombe and Phelps [333] concluded that the average molecule in the PPL complex consisted of four or five protein cores associated with 100 chains of chondroitin sulphate which tended to form aggregates in which keratan sulphate and glycoprotein were involved. Proteolytic enzymes easily penetrated the spaces created by random aggregation, and cleavage of a few bonds resulted in a rapid disintegration of the whole complex.

Biosynthesis of Sulphated Glycosaminoglycans.—Previous work has established that adenosine 3-phosphate 5-phosphosulphate (PAPS) is a key

[333] M. Luscombe and C. F. Phelps, *Biochem. J.*, 1967, **103**, 103.

intermediate in the transfer of sulphate to highly polymerised glycosaminoglycans or precursors of smaller molecular weight. The enzymic preparation of PAPS labelled with ^{35}S has been reported.[334] A microsomal preparation from mouse mast-cell tumours catalysed both the incorporation of $^{35}SO_4^{2-}$ from [^{35}S]PAPS into microsomal heparin [335] and the incorporation of sugars into a microsomal *N*-acetylated glycosaminoglycan related to heparin.[336] The same preparation catalysed the incorporation of $^{35}SO_4^{2-}$ into the newly formed glycosaminoglycan to give products related to heparin but containing varying degrees of sulphation.[337] In the latter reaction *N*-deacetylation of the glycosaminoglycan occurred to the same extent as *N*-sulphation.[338] It has been suggested [337, 338] that in the formation of sulphated glycosaminoglycan, sulphation can take place to an advanced degree before total polymerisation and that sulphation of sugar residues may occur as they are added sequentially to the glycosaminoglycans.

An enzyme which catalysed the transfer of sulphate from PAPS primarily to the amino-groups of *N*-desulphated heparin has been purified from mouse mast-cell tumour.[339] However, Eisenman *et al.*[339] endorsed an earlier hypothesis [340] that, in heparin biosynthesis, sulphate transfer occurred subsequent to polymerisation of the polysaccharide chain, rather than to small molecular weight precursors.

A diverse group of polysaccharides was capable of accepting sulphate from a glycosaminoglycan-sulphating system isolated from the 105,000 × g supernatant from homogenates of chick embryo cartilage.[341] The specificity of the sulphating system *in vivo*, however, might depend upon the intact protein complex. Polysaccharides with equimolar proportions of sulphate and uronic acid were the best sulphate acceptors *in vitro*. The endogenous sulphate acceptor present in boiled, dialysed preparations of the supernatant, which also contained the sulphating system, was a molecule of the chondroitin sulphate type [342] whose ability to accept sulphate was strongly influenced by its charge properties rather than by its molecular size. The primary site of sulphation was the axial hydroxy-group at C-4 of a 2-acetamido-2-deoxygalactosyl moiety, but pretreatment of the acceptor with pronase or alkali resulted in sulphation predominantly of an equatorial hydroxy-group, which suggested that the protein portion of the protein–polysaccharide complex might determine the site of sulphation. A mechanism in which sulphation occurred more or less concurrently with

[334] A. S. Balasubramanian, L. Spolter, L. I. Rice, J. B. Sharon, and W. Marx, *Analyt. Biochem.*, 1967, **21**, 22.
[335] J. E. Silbert, *J. Biol. Chem.*, 1967, **242**, 2301.
[336] J. E. Silbert, *J. Biol. Chem.*, 1963, **238**, 3542.
[337] J. E. Silbert, *J. Biol. Chem.*, 1967, **242**, 5146.
[338] J. E. Silbert, *J. Biol. Chem.*, 1967, **242**, 5153.
[339] R. A. Eisenman, A. S. Balasubramanian, and W. Marx, *Arch. Biochem. Biophys.*, 1967, **119**, 387.
[340] E. A. Davidson and K. Meyer, *J. Biol. Chem.*, 1954, **211**, 605.
[341] E. Meezan and E. A. Davidson, *J. Biol. Chem.*, 1967, **242**, 1685.
[342] E. Meezan and E. A. Davidson, *J. Biol. Chem.*, 1967, **242**, 4956.

polysaccharide elongation rather than on a preformed polysaccharide was consistent with the experimental results. A similar mechanism for sulphation in heparin biosynthesis has already been mentioned.[337, 338]

An enzyme purified from homogenates of the albumen-secreting region of hen oviduct catalysed the transfer of sulphate from PAPS to C-6 of the 2-acetamido-2-deoxygalactosyl moiety of UDP 2-acetamido-2-deoxygalactose 4-sulphate.[343]

Other Sulphated Glycosaminoglycans

Glucan sulphate isolated from the hypobranchial mucin of the whelk (*Buccinum undatum* L.) was reported [344] to have a cellulose-like structure. In biosynthesis of the glucan sulphate, sulphation preceded or closely accompanied polymerisation. A neutral polysaccharide was also obtained from the same source.[345] Comparative analyses of these materials isolated from three species (*Buccinum undatum*, *Neptunea antiqua*, and *Pecten maximus*) indicated [345] that the systems were similar but differed markedly in the ratio of charged to uncharged polysaccharide and that the composition of the neutral material varied considerably. For example, the molar ratio of 2-amino-2-deoxyhexose to glucose in the three species mentioned above was 1 : 16, 1 : 4, and 1 : 0·6 respectively, and the approximate ratios of 2-amino-2-deoxyglucose to 2-amino-2-deoxygalactose were 1 : 1, 2 : 3, and 1 : 1 respectively.

A new group of vertebrate acidic glycosaminoglycans, designated Lorenzan sulphates, has been isolated [346] from the glands of Lorenzini, a sense organ, in elasmobranch fish. The material isolated from *Squalus acanthias* by papain digestion and purification on Sephadex and DEAE-cellulose was shown to be a sulphated glycosaminoglycan containing 2-acetamido-2-deoxyglucose, 2-acetamido-2-deoxygalactose, and galactose. The composition of these molecules varied with the animal species.[347]

An antigenic glycosaminoglycan has been isolated from the allantoic fluid of embryonated eggs, either normal or infected with influenza virus.[348,349] Analyses of the purified allantoic antigen [348] and of a fraction obtained after proteolytic digestion [350] are summarised in Table 3.

Structural studies of the proteolytically digested antigen indicated [350] a molecule in which a peptide backbone was substituted by multiple, short

[343] T. Harada, S. Shimizu, Y. Nakanishi, and S. Suzuki, *J. Biol. Chem.*, 1967, **242**, 2288.
[344] S. Hunt and K. M. Rudall, *Carbohydrate Res.*, 1967, **4**, 259.
[345] J. Doyle, *Biochem. J.*, 1967, **103**, 41P.
[346] J. Doyle, *Biochem. J.*, 1967, **103**, 325.
[347] J. Doyle, *Comp. Biochem. Physiol.*, 1963, **24**, 479.
[348] G. Haukenes, A. Harboe, and K. Mortensson-Egnund, *Acta Path. Microbiol. Scand.*, 1966, **66**, 510.
[349] A. Harboe and G. Haukenes, *Acta Path. Microbiol. Scand.*, 1966, **68**, 98 and refs. cited therein.
[350] K. Meyer, V. P. Bhavanandan, D. Yung, L. T. Lee, and C. Howe, *Proc. Nat. Acad Sci. U.S.A.*, 1967, **58**, 1655.

chains of carbohydrate attached by *O*-glycosidic linkages mainly between 2-acetamido-2-deoxygalactose and serine or threonine. In contrast to keratan sulphate, however, the 2-acetamido-2-deoxygalactosyl residues

Table 3. *Chemical analyses of allantoic fluid glycosaminoglycan*

Component (%)	*Whole antigen*	*Antigen after proteolytic digestion*
	Ref. 348	*Ref.* 350
Galactose	27·0	28·1
2-Amino-2-deoxyglucose	19·5	11·9
2-Amino-2-deoxygalactose	9·0	8·8
Sulphate	12·0	6·0
Sialic acid	*	12·7
Fucose	4·3	*
Acetyl	*	*
Total protein[a]		11·1
Amino-acids (as % total protein)		
Threonine		27·0
Serine		27·9
Glutamic acid		4·6
Aspartic acid		0·8
Proline		17·6
Alanine		5·9
Glycine		0·7
Isoleucine		2·6
Leucine		6·6
Valine		3·8

* Not determined.
[a] By amino-acid analyser.

were probably not substituted at C-3. The sulphate ester in the allantoic antigen was mainly as 2-acetamido-2-deoxyglucose 6-sulphate.

Polysaccharide Sulphates and other Polysaccharides from Seaweeds

Fractionation studies with potassium chloride have shown that the three different carrageenans isolated from *Chondrus crispus*, *Gigartina stella*, and *G. skottsbergii* are series of polysaccharides of different chemical composition, and hence of different solubility, and are much more complex than was indicated by the classical κ and λ picture.[351] There was a close correlation between the content of 3,6-anhydro-D-galactose and the potassium chloride concentration at which the carrageenan became insoluble. These conclusions were endorsed by structural investigations.

κ-Carrageenan from *G. skottsbergii* was resolved into four subfractions by addition of potassium chloride.[352] Studies of two very similar fractions showed that for every four galactosyl and every three 3,6-anhydrogalactosyl residues the polysaccharide contained five sulphate groups, two of which were present as galactose 4-sulphate, two as 3,6-anhydrogalactose

[351] A. J. Pernas, O. Smidsrød, B. Larsen, and A. Haug, *Acta Chem. Scand.*, 1967, **21**, 98.
[352] A. S. Cerezo, *J. Chem. Soc.* (*C*), 1967, 992.

2-sulphate, and the fifth as galactose 6-sulphate. All the galactosyl residues were (1 → 3)-linked except the residue of galactose 6-sulphate which was probably (1 → 4)-linked. The κ-carrageenans probably contained trace amounts of xylose.[352] The carrageenan fraction precipitated at a 0·3–0·4M-potassium chloride concentration contained for every ten (1 → 3)-linked galactosyl residues, six (1 → 4)-linked 3,6-anhydrogalactosyl residues, and ten sulphate groups, five as 3,6-anhydrogalactose 2-sulphate and five as galactose 2-sulphate.[353]

κ-Carrageenan from the red seaweed *Gigartina tenella* contained D-galactose, 3,6-anhydro-D-galactose, and sulphate in the molar proportions 1 : 0·98 : 1·17 and small amounts of L-galactose and D-xylose.[354] Partial methanolysis of the polysaccharide showed that carrabiose [4-*O*-(β-D-galactopyranosyl)-3,6-anhydro-D-galactose] was the chief repeating unit of the polysaccharide. Carrabiose, synthesised [355] by a condensation reaction of methyl-3,6-anhydro-2-*O*-toluene-*p*-sulphonyl-α-D-galactopyranoside with tetra-*O*-acetyl-α-D-galactopyranosyl bromide and subsequent removal of blocking groups, was identical with the product isolated from natural sources.

A sulphated polysaccharide, designated aeodan, extracted from the red seaweed *Aeodes orbitosa* with hot water contained D-galactose, 2-*O*-methyl-D-galactose, and sulphate ($NaSO_3^-$) in the molecular proportions 12 : 2 : 9, in addition to trace amounts of D-xylose and glycerol.[356] Structural studies of aeodan and desulphated aeodan by periodate oxidation, methylation, and alkaline degradation indicated the presence of (1 → 3)- and (1 → 4)-linked residues with sulphate at C-2 and C-6. A sulphated polysaccharide extracted similarly from the red seaweed *Grateloupia elliptica* and purified by precipitation as its cetyl pyridinium salt contained [357] D-galactose, L-galactose, 3,6-anhydro-D-galactose, and sulphate in the molar proportions 9 : 1 : 2 : 8. Small amounts of D-xylose, 2-*O*-methyl-L-galactose, 4-*O*-methyl-D-galactose, and 2-*O*-methyl-3,6-anhydro-L-galactose were also isolated and crystallised from polysaccharide hydrolysates. The partially methylated sugars have seldom or never been found in polysaccharides.

Hydrolytic enzymes, isolated from a sea-water bacterium classified as a *Cytophaga* sp. (order *Myxobacteriales*), catalysed the hydrolysis of the algal galactan sulphate, porphyran, in the region of the residues of 3,6-anhydro-L-galactose.[358] The degradation products included D-galactose, 6-*O*-methyl-D-galactose, neoagarobiose (3,6-anhydro-*O*-α-L-galactopyranosyl-(1 → 3)-D-galactose), neoagarotetraose (4^2-β-neoagarobiosylneoagarobiose), and oligosaccharides containing 6-*O*-methyl-D-galactose, the major of which was tentatively identified as 6^3-*O*-methylneoagarotetraose:

[353] A. S. Cerezo, *J. Chem. Soc.* (*C*), 1967, 2491.
[354] S. Hirase and K. Watanabe, *Bull. Chem. Soc. Japan*, 1967, **40**, 1442.
[355] S. Hirase and C. Araki, *Bull. Chem. Soc. Japan*, 1967, **40**, 2627.
[356] J. R. Nunn and H. Parolis, *Carbohydrate Res.*, 1968, **6**, 1.
[357] S. Hirase, C. Araki, and K. Watanabe, *Bull. Chem. Soc. Japan*, 1967, **40**, 1445.
[358] J. R. Turvey and J. Christison, *Biochem. J.*, 1967, **105**, 311.

sulphated fragments were also isolated but not identified.[359] It was suggested [359] that porphyran contained some structural sequences identical with those found in agarose, and other portions in which 6-*O*-methyl-D-galactosyl units replaced some of the D-galactosyl units. Some of the 6-*O*-methyl-D-galactose might occur as single units flanked by D-galactose and the anhydro-sugar. Since *ca.* 70% of the porphyran was recovered as high molecular weight fragments it was suggested that the 6-*O*-methyl ether or sulphate functions, that acted as barriers to enzyme actions, were distributed throughout the molecule.

Aqueous extracts of the brown seaweed *Ascophyllan nodosum* contained, in addition to alginate, three sulphated glucuronofucans.[360] The predominant member of this group, designated ascophyllan, was a polyglucuronide to which were attached side-chains composed of residues of D-xylose, L-fucose, and sulphate. Isolation and characterisation of a disaccharide as 3-*O*-(β-D-xylopyranosyl)-L-fucose ($[\alpha]_D - 66{\cdot}5 \pm 2°$) suggested [360] that ascophyllan did not consist of separate chains of the fucoidan and xylan types linked to the polyuronide backbone. A cell-wall glucuronofucan of more complex structure has been isolated from *Ascophyllum nodosum* and *Laminaria hyperborea* after removal of laminarin, fucoidan, and alginate.[361] Treatment of the weed residues with formaldehyde to complex phenolic material, followed by extraction with ammonium oxalate–oxalic acid buffer (pH 2·8) at 80°, gave the polysaccharide ($[\alpha]_D - 225°$, L-fucose 49%, D-xylose 10%, D-glucuronate sodium salt 12%, sodium hydrogen sulphite 21%, and protein 4%) in 32% yield based on the weed residue. Fractionation failed to produce a homopolymer. Partial hydrolysis with a water-soluble, acidic resin liberated L-fucose, D-xylose, 3-(or 4)-*O*-(D-xylosyl)-L-fucose ($[\alpha]_D - 48°$), a fucosyl-xylose, and three oligosaccharides containing varying proportions of fucose, xylose, glucuronic acid, and sulphate, together with degraded polysaccharide (17%, $[\alpha]_D - 87°$). 2-*O*-(α-D-Glucuronosyl)-L-fucose was characterised as a product of hydrolysis with 2N-oxalic acid at 100°.

Agar.—Small amounts of D-xylose, 6-*O*-methyl-D-galactose, and 4-*O*-methyl-L-galactose in addition to D- and L-galactose have been isolated [362] as the crystalline sugars from hydrolysates of a commercial agar and from the agar of *Gelidium amansii*. The commercial preparation also liberated an *O*-methyl-pentose on hydrolysis. An acid-resistant, reducing disaccharide, designated as isoagarobiose, present in 1·5% yield in a total acid hydrolysate of commercial agar has been identified [363] as a reversion product. Its structure was tentatively postulated as 4,6-*O*-(3′,6′-anhydro-*aldehydo*-L-galactosylidene)-D-galactose from the results of methanolysis and methylation studies.

[359] J. R. Turvey and J. Christison, *Biochem. J.*, 1967, **105**, 317.
[360] B. Larsen, *Acta Chem. Scand.*, 1967, **21**, 1395.
[361] E. Percival, *Chem. and Ind.*, 1967, 511.
[362] C. Araki, K. Arai, and S. Hirase, *Bull. Chem. Soc. Japan*, 1967, **40**, 959.
[363] C. Araki and K. Arai, *Bull. Chem. Soc. Japan*, 1967, **40**, 1452.

The 4,6-*O*-(1-carboxyethylidene)-D-galactopyranosyl residues in agar have been shown to contain equatorial *C*-methyl groups.[136, 137]

Laminarin.—Enzymic degradation of soluble laminarin from *Laminaria saccharina* produced, in addition to D-glucose and laminaribiose, two reducing and two nonreducing oligosaccharides.[364] The latter were tentatively identified as laminaribiosylmannitol and β-D-glucosyl mannitol. The reducing oligosaccharides were 6^2-β-D-glucosyl-laminaribiose and a structurally related tetrasaccharide that contained a (1 → 6)-linked D-glucosyl residue.

Fucoidan.—A partially purified α-L-fucosidase isolated from the hepatopancreas of abalone (*Haliotus* sp.) hydrolysed the algal polysaccharide fucoidan, an α-(1 → 2)-linked polyfucose 4-sulphate, without desulphation to give oligosaccharides, containing 2–10 L-fucose units as well as L-fucose.[365]

Alginic Acid.—Intramolecular catalysis involving the carboxy group has been postulated[366] as the main mechanism responsible for the hydrolysis of alginates at pH values above 2. The rates of acid-catalysed hydrolysis of β-D-glucopyranosiduronic acids containing aglycones of different affinity were inversely proportional to the electron affinity of the aglycone, whereas the rates of hydrolysis of the corresponding glucosides were independent of the polarity of the aglycone.[367] The energies and entropies of activation of the β-D-glucopyranosiduronic acid derivatives were lower than those of the corresponding β-D-glucopyranosides.

Studies on the chemical structure and physical properties of alginates showed that the strontium selectivity of alginates was due to their content of residue of L-guluronic acid,[368] and that the higher solubility in acid of alginates from *Ascophyllum nodosum* as compared with those from *Laminaria hyperborea* was related to the presence of smaller proportions of homopolymer blocks in the *Laminaria* alginates.[369] The electrophoretic mobility of alginate fractions in electrolytes containing sodium and calcium ions depended upon their uronic acid composition.[370] Electrophoretic analysis showed that both heterogeneous and homogeneous hydrolysis by acid split the alginate from *Laminaria digitata* into chemically different fragments. It was concluded[370] that the alginic acid molecule was a block polymer which contained long sequences of predominantly alternating structure (guluronic acid–mannuronic acid) and long sequences of both gulosyluronic acid and mannosyluronic acid residues. An oligouronoside with DP of 13 and containing 97% of L-guluronic acid has been isolated[371] from a commercial alginate by use of selective precipitation and partial hydrolysis with acid.

[364] M. Fleming, D. J. Manners, and A. J. Mason, *Biochem. J.*, 1967, **104**, 32P.
[365] N. M. Thanassi and H. I. Nakada, *Arch. Biochem. Biophys.*, 1967, **118**, 172.
[366] O. Smidsrød, B. Larsen, and A. Haug, *Carbohydrate Res.*, 1967, **5**, 371.
[367] M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, 1967, **5**, 453.
[368] A. Haug and O. Smidsrød, *Nature*, 1967, **215**, 757.
[369] A. Haug, S. Myklestad, B. Larsen, and O. Smidsrød, *Acta Chem. Scand.*, 1967, **21**, 768.
[370] A. Haug B. Larsen, and O. Smidsrød, *Acta Chem. Scand.*, 1967, **21**, 691.
[371] E. R. Humphreys, *Carbohydrate Res.*, 1967, **4**, 507.

Minor features and structural variations of the nine alginate samples isolated from different seaweeds, and varying in their relative contents of mannuronic and guluronic acids, have been determined [372] by methylation, reduction, and hydrolysis. All the polysaccharides were shown to be linear and to contain no (1 → 3)- or (1 → 2)-linkages. The units in alginate which resisted oxidation with periodate were (1 → 4)- or (1 → 5)-linked and a physical, rather than a chemical, explanation has been postulated to explain this periodate resistance.

Comparison of the percentage reduction of the functional groups of alginic acid and its esters with diborane and with lithium borohydride showed that the order of reactivity towards the latter reducing agent was hemi-acetal end group > propionyl ester group > uronic acid carboxy-group.[373] The lithium borohydride procedure has been recommended for the reduction of acidic polysaccharides since it reduced all the esterified carboxy groups and all the hemiacetal groups, without ether formation.

Oxidative–reductive Depolymerisation of Alginate, Hyaluronate, and Other Polysaccharides

In the ascorbic acid-induced depolymerisation of hyaluronic acid, as measured by viscometry, degradation of diketogulonic acid was one of the major reactions to which the depolymerisation of hyaluronic acid was coupled.[374]

No changes were observed [375] in the i.r. spectrum, specific optical rotation ($[\alpha]_D^{20} = -74°$), or contents of glucuronic acid and 2-amino-2-deoxy-glucose of a sample of hyaluronic acid following treatment with ascorbic acid. The amino-acid content, however, was reduced by 30% by such treatment, and it was suggested that the site of the reaction of ascorbic acid might be located in the protein moiety of the macromolecule. Studies of the viscometric behaviour of sodium alginate, propene glycol alginate, hyaluronic acid, polyacrylic acid, polymethacrylic acid, soluble starch, dextrin, glycogen, dextran, methyl cellulose, and a co-polymer of methyl vinyl ether and maleic anhydride in the presence of L-ascorbic acid and, in some cases, L-cysteine or ferrous ion showed that the viscosity of all the polyelectrolytes decreased in the presence of autoxidants, but that neutral polysaccharides generally showed no change in viscosity.[376] Subsequent reports [377] showed that conclusions about the relative rates of depoly-merisation of different polysaccharides could not be made from viscosity studies alone unless the relationship between the intrinsic viscosity and degree of polymerisation were considered. Thus, measurements of increases

[372] D. A. Rees and J. W. B. Samuel, *J. Chem. Soc.* (*C*), 1967, 2295.
[373] J. H. Manning and J. W. Green, *J. Chem. Soc.* (*C*), 1967, 2357.
[374] W. Niedermeier, C. Dobson, and R. P. Laney, *Biochim. Biophys. Acta*, 1967, **141**, 366.
[375] D. A. Swann, *Biochem. J.*, 1967, **102**, 42C.
[376] A. Herp, T. Rickards, G. Matsumura, L. B. Jakosalem, and W. Pigman, *Carbohydrate Res.*, 1967, **4**, 63.
[377] O. Smidsrød, A. Haug, and B. Larsen, *Carbohydrate Res.*, 1967, **5**, 482.

in reducing power established that the rates of oxidative–reductive depolymerisation were approximately the same for neutral polysaccharides and polysaccharides containing carboxy-groups.

It has been reported [378] that the oxidative dissociation of *O*-glucuronides by L-ascorbic acid and catalytic amounts of cupric ion was effected by hydrogen peroxide derived from the ascorbic acid. Hydrogen peroxide in the presence of Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Mn^{2+}, or Zn^{2+} ions produced a similar dissociation of *O*-glucuronides.

[378] Y. Yamane, K. Sakai, and K. Ikeguchi, *Yakugaku Zasshi*, 1967, **87**, 227.

7
Miscellaneous Polysaccharides

Onuphic acid, a polymer isolated from the tube of the polychaete *Hyalinocia tubicola*, was shown [379] to contain D-glucose, L-fucose, phosphomonoester, and phosphodiester in the ratios 22 : 1 : 25 : 3. Periodate oxidation and hydrolysis studies indicated a structure of (1 → 3)-linked glucosyl residues, phosphorylated at C-4 and C-6, to which fucosyl residues were attached probably through phosphodiester linkages.

A high molecular weight fraction extracted from an organic soil with dilute acid has been separated into four polysaccharide fractions by anion-exchange chromatography.[380] Each fraction contained arabinose, galactose, glucose, mannose, L-rhamnose, xylose, and glucuronic acid in different proportions and the most acidic fraction contained an unidentified acidic component which is not a uronic acid.

A galactan, $[\alpha]_D^{20}$ +20·5°, average molecular weight $2{\cdot}2 \times 10^6$, has been isolated [381] from homogenates of the eggs of the pond snail (*Hymnaea stagnalis*).

A highly branched galactan isolated from the albumen gland of the mollusc *Biomphalaria glabrata* contained D- and L-galactosyl residues (64% and 36%, respectively) linked β-(1 → 3) and β-(1 → 6) in approximately the same proportions.[382] Equal numbers of D- and L-galactosyl end-groups were present. The disaccharides 6-*O*-(D-galactopyranosyl)-D-galactopyranose and 3-*O*-(D-galactopyranosyl)-D-galactopyranose were liberated on mild hydrolysis of the galactan with acid. The structural evidence suggested that the galactan was similar to that isolated from another gastropod.[383a]

[379] F. G. E. Pautard and H. Zola, *Carbohydrate Res.*, 1967, **3**, 271; **4**, 78.
[380] S. A. Barker, M. H. B. Hayes, R. G. Simmonds, and M. Stacey, *Carbohydrate Res.*, 1967, **5**, 13.
[381] H. Fleitz and H.-J. Horstmann, *Z. physiol. Chem.*, 1967, **348**, 1301.
[382] J. B. C. Corrêa, A. Dmytraczenko, and J. H. Duarte, *Carbohydrate Res.*, 1967, **3**, 445.
[383a] P. O'Colla, *Proc. Roy. Irish Acad., B*, 1952, **55**, 165.

8

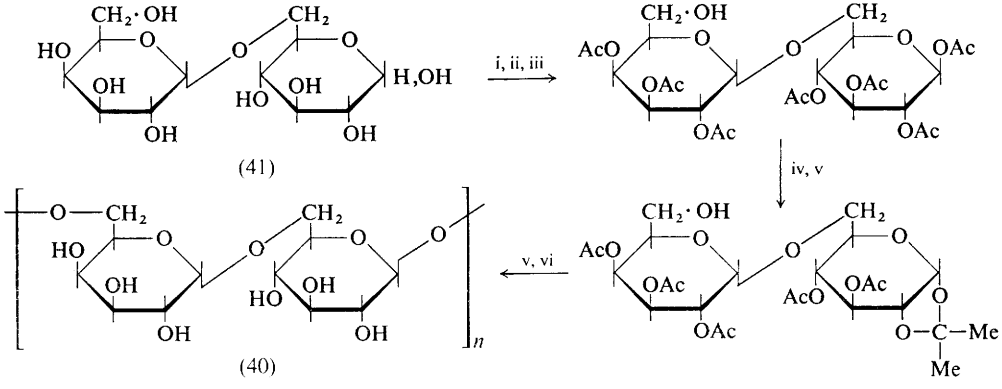
Chemical Synthesis and Modification of Polysaccharides

The chemical synthesis of polysaccharides of defined structure was the subject of a recent review.[383b]

The synthesis[384] of a regular heteropolysaccharide (40) involving the polycondensation of a disaccharide orthoester derived from melibiose (41) is summarised below. A polysaccharide fraction free from oligosaccharides was obtained by sequential gel chromatography on Sephadex G-25 and precipitation with alcohol. Structural studies confirmed that the product was a regular heteropolysaccharide with alternating (1 → 6)-linked residues of β-D-glucopyranose and α-D-galactopyranose units. Polycondensation reactions of monosaccharides to give homopolymers and a series of oligosaccharides (DP 2–10) have been reported[385] using phosphorus compounds as condensing agent and formamide, DMF, and DMSO as solvents. Two nondialysable, branched homopolymers, a xylan, and a galactan, synthesised in the presence of phosphorus pentoxide in DMSO at 40°, have been partially characterised.[386] Polymerisation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose in the presence of phosphorus pentafluoride and subsequent debenzylation gave a poly α-(1 → 6)-anhydro-D-glucopyranose of high polymeric character and stereoregularity.[387]

It has been shown[388] that the polycondensation of the anomers of D-glucose, D-galactose, and D-mannose and some of their derivatives in the molten state at temperatures below 220° was predominantly an intermolecular condensation reaction which occurred primarily between C-1 and C-6. The hydroxy-group at C-1 was essential to condensation. Under standard conditions the extent of polymerisation was greatest for the D-galactoses followed by the D-mannoses, with the D-glucoses giving the smallest polymers. The α-anomers of D-galactose and D-mannose gave products with a higher degree of polymerisation than those obtained from

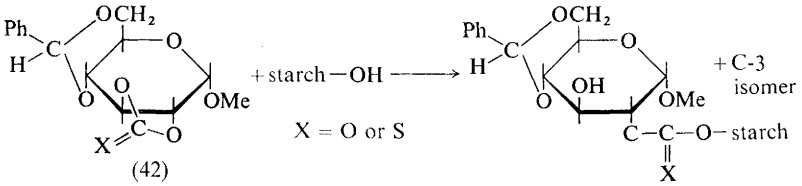
[383b] I. J. Goldstein and T. L. Hullar, *Adv. Carbohydrate Chem.*, 1966, **21**, 431.
[384] N. K. Kochetkov, A. F. Bochov, and I. G. Yazlovetsky, *Carbohydrate Res.*, 1967, **5**, 243.
[385] T. Mizuno, *J. Agric. Chem. Soc. Japan*, 1967, **41**, 189.
[386] T. Mizuno, *J. Agric. Chem. Soc. Japan*, 1967, **41**, 195.
[387] E. R. Ruckel and C. Schuerch, *Biopolymers*, 1967, **5**, 515.
[388] J. W. Liskowitz and B. Carroll, *Carbohydrate Res.*, 1967, **5**, 245.



Reagents: i, tritylation; ii, acetylation; iii, H^+; iv, $AlCl_3$; v, MeOH, 2,6-dimethylpyridine; vi, $HgBr_2$; vii, NaOMe.

the β-D-anomers. At temperatures above 220° intramolecular reactions became more apparent.

A reaction sequence has been described[389] for the substitution on starch of simple pyranoid sugars possessing vicinal *trans*-hydroxy-groups by reaction of the *trans*-fused cyclic carbonate or thionocarbonate (42) of the sugar with starch in the presence of triethylamine at 25°.



(42)

The reactivities of the hydroxy-groups of sugar residues have been studied by analyses of the esterification products of various homopolysaccharides. Homogeneous formylation of the primary hydroxy-groups in dextrin, a low molecular weight polymer containing (1 → 4)-linked α-D-glucopyranosyl residues, was thermodynamically favoured.[390] The two secondary hydroxy-groups had quite different reactivities; one, probably the C-2-hydroxy-group, was esterified rapidly to a low level of equilibrium, the other was slowly esterified to a higher level of equilibrium. Analyses of the hydrolysis products from reactions of cellulose with 2-diethylamino-ethyl chloride in aqueous and nonaqueous media showed[391] that the relative distribution of the 2-diethylamino-ethyl groups on the C-2, C-3, and C-6-hydroxy-groups in the mono-*O*-(2-diethylamino-ethyl)-D-glucopyranose fraction of the hydrolysate was independent of the degree of substitution. The relative distribution of the 2-diethylamino-ethyl groups among the 2-*O*-, 3-*O*-, and 6-*O*-D-glucopyranosyl residues of cotton cellulose following reaction with 2-diethylamino-ethyl chloride in excess of sodium hydroxide at room temperature was found to be 1·27 : 0·35 : 1·00.[392] By comparison, the treatment of cellulose with 2-diethylamino-ethyl chloride in 1,4-dioxan gave a higher ratio of substitution on the C-2 and C-3-hydroxy-groups relative to the C-6-hydroxy-group.[391] Increased substitution on the C-2-hydroxy-group was accompanied by a decrease in substitution at the C-6-hydroxy-group with little change in the amount of substitution at the C-3-hydroxy-group. Reactions of starch followed a similar pattern to those of cellulose, but the increase in substitution on both the C-2 and C-3-hydroxy-groups relative to that on the C-6-hydroxy-group was more pronounced than for cellulose. With respect to the change

[389] W. M. Doane, E. I. Stout, B. S. Shasha, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1967, **5**, 366.
[390] S. P. Rowland, M. A. F. Brannan, H. J. Janssen, and P. F. Pittman, *Carbohydrate Res.*, 1967, **3**, 361.
[391] E. J. Roberts and S. P. Rowland, *Carbohydrate Res.*, 1967, **5**, 1.
[392] E. J. Roberts and S. P. Rowland, *Canad. J. Chem.*, 1967, **45**, 261.

in the fraction of substitution occurring at the C-3-hydroxy-group with changes of the reaction conditions, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside behaved similarly to starch, whereas the β-D-anomer behaved similarly to cellulose.

Esterification of cotton cellulose with propionyl chloride in pyridine and DMF gave a product [393] in which approximately one out of every four hydroxy-groups was substituted with an α-propionylpropionyl residue; the rest with propionyl residues. Such side-reactions took place at early stages in the esterification process and only in the presence of organic bases such as pyridine.

Reaction of sulphur trioxide with DMSO produced a stable, solid complex which sulphated cellulose to degrees of substitution of 1·3–2·0 in 15–30 min. at 15–25°.[394] The product precipitated protein from aqueous solutions.

Amylose aminated at the secondary hydroxy-groups has been prepared [395] by sequential tritylation, toluene-*p*-sulphonylation, reaction with sodium azide or hydrazine, reduction, and detritylation. Attempts to aminate amylose and 6-*O*-tritylamylose by sequential oxidation with periodate, controlled reaction with phenylhydrazine, and reduction failed at the reduction stage.

Several phosphorus-containing derivatives of starch have been prepared [396] by treating corn starch with chloromethylphosphonic dichloride in pyridine.

The results of dextrinisation of amylopectin in the presence of uniformly labelled D-[^{14}C]glucose or 2,3,6-tri-*O*-methyl-D-glucose strongly supported codextrination and the incorporation of new monomeric units into a dextrinised polysaccharide.[397]

[393] A. K. Sircar, D. J. Stanonis, and C. M. Conrad, *J. Appl. Polymer Sci.*, 1967, **11**, 1683.
[394] R. L. Whistler, A. H. King, G. Ruffini, and F. A. Lucas, *Arch. Biochem. Biophys.*, 1967, **121**, 358.
[395] M. L. Wolfrom, H. Kato, M. I. Taka, A. Sato, G. U. Yuen, T. Kinoshita, and E. J. Soltes, *J. Org. Chem.*, 1967, **32**, 3086.
[396] F. R. T. Rosenthal, A. M. N. Corrêa, and E. Tolmasquim, *Cereal Chem.*, 1967, **44**, 554.
[397] M. H. Fischer and F. Smith, *Cereal Chemistry*, 1967, **44**, 551.

9
Physicochemical Studies

Studies of the aggregation of cationic dyes on various classes of acidic polysaccharides in dilute solution have permitted the determination of equivalent weights using approximately 25 μg. of polymer.[398] In addition, the titration curves obtained with Methylene Blue and proflavine provided a spectrophotometric means of distinguishing between acidic polysaccharides with relatively homogeneous and nonhomogeneous charge distributions. Studies of the binding of purified collagen with heparin and chondroitin sulphate showed [399] that the binding curves, which related the amount of polyanion fixed as a function of pH, had similar shapes to the acid titration curves of collagen. The concentrations of salt required to dissolve the complexes formed between acidic glycosaminoglycans and proteins have been shown [400a] to increase with increasing content of sulphate ester and decreasing pH and the presence of such complexes might prevent histological recognition of the components.

Studies of the interaction of chondroitin sulphate with solutions containing calcium and phosphate ions suggested that,[400b] in calcifying cartilage or epiphyseal plate, there were steps or compartments in the concentration gradients of calcium and phosphate between blood and solid mineral. Specific interactions of some cartilage protein polysaccharides with freshly precipitating calcium phosphate have been demonstrated.[401]

An elegant method has been reported [402] for the study of the interactions of polyanions with cationic counter-ions. Thus, the rate of reaction of Methylene Blue with hydrated electrons produced in pulse radiolysis, which produced Methylene Blue semiquinone, was considerably slower in the presence of polyanions such as chondroitin sulphates. In the presence of agents which would break down the complex formed between Methylene Blue and polyanion, the rate of the reaction leading to the production of the semiquinone was similar to that observed in the absence of polyanion. Changes in the rate of the reaction of the hydrated electron could be used

[398] A. L. Stone and D. F. Bradley, *Biochim. Biophys. Acta*, 1967, **148**, 172.
[399] A. Serafini-Fracassini, *Biochem. J.*, 1967, **104**, 13P.
[400a] J. E. Scott, *Biochem. J.*, 1967, **104**, 14P.
[400b] J. M. Bowness and K. H. Lee, *Biochem. J.*, 1967, **103**, 382.
[401] J. Di. Salvo and M. Schubert, *J. Biol. Chem.*, 1967, **242**, 705.
[402] J. S. Moore, G. O. Phillips, K. S. Dodgson, and J. V. Davies, *Biochem. J.*, 1967, **104**, 18P.

as a specific indication of interactions between dye and anionic sites on macromolecules.

The structures of amylose triacetate,[403] V amyloses,[404] and the complexes of amylose with DMSO [405] and with halogen-substituted hydrocarbons [406] have been investigated by *X*-ray crystallographic studies. The molecular configuration of amylose and its complexes with iodine in aqueous solution has been reported [407] and complex formation between solid amylose and small molecular weight organic molecules has been studied [408] by gas–liquid chromatographic methods. Measurements of the intrinsic viscosities and diffusion coefficients of the cellodextrins cellobiose to cellohexaose have been used [409] to determine the shape of these molecules in aqueous solution at 25–70°. Thermodynamic and kinetic studies of the formation of inclusion compounds of α-cyclodextrins with various dyes have been reported [410] and it has been proposed that the dyes were enclosed in the cyclodextrin ring. The cyclic carbohydrates cyclohexa-, cycloheptа-, and cyclo-octa-amylose have been shown to interact with potato phosphorylase.[411]

Studies of the formation of complexes between gelatin and various dextrans of known structure suggested [412] that the terminal, nonreducing groups of the polysaccharides were probably not the active binding site with gelatin. Such residues were considered to be important, however, in the formation of complexes between glucans and the protein concanavalin A. (see also p. 207).

[403] A. Sarko and R. H. Marchessault, *J. Amer. Chem. Soc.*, 1967, **89**, 6454.
[404] H. F. Zobel, A. D. French, and M. E. Hinkle, *Biopolymers*, 1967, **5**, 837.
[405] A. D. French and H. F. Zobel, *Biopolymers*, 1967, **5**, 457.
[406] R. R. Bumb and B. Zaslow, *Carbohydrate Res.*, 1967, **4**, 98.
[407] T. Szejlti, S. Augustat, and M. Richter, *Biopolymers*, 1967, **5**, 5, 17.
[408] T. Kuge and K. Takeo, *Agric. and Biol. Chem. (Japan)*, 1967, **31**, 257.
[409] M. Ihnat and D. A. I. Goring, *Canad. J. Chem.*, 1967, **45**, 2353, 2363.
[410] F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Amer. Chem. Soc.*, 1967, **89**, 14.
[411] J. Staerk and H. Schlenk, *Biochim. Biophys. Acta*, 1967, **146**, 120.
[412] R. J. Doyle, E. E. Woodside, and C. W. Fischel, *Carbohydrate Res.*, 1967, **5**, 274.

10
Glycolipids

Analytical and degradative studies have indicated[413] that a new glycolipid sulphate ester from the extremely halophilic bacterium *Halobacterium cutirubrum* had one of the two following structures (43) or (44). Three glycolipids isolated from *Arthrobacter globiformis* 616 have been characterised[414] as *O*-β-D-galactopyranosyl-(1 → 1)-glycerol, *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 1)-glycerol, and *O*-α-D-mannopyranosyl-(1 → 3)-*O*-α-D-mannopyranosyl-(1 → 1)-glycerol. Saponification of the glycolipid of *Staphylococcus lactis* 13 produced a glycoside characterised[415] as *O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 1)-D-glycerol. Glycerogalactolipids have also been found in sheep brain.[416]

$^{-}O_3SO$-3-Gal 1 → 6 Man 1 → 2G 1 → 1′-2′,3′-di-*O*-phytanyl-L-glycerol

(43)

$^{-}O_3SO$-3-Gal 1 → 2G 1 → 6 Man 1 → 1′-2′,3′-di-*O*-phytanyl-L-glycerol

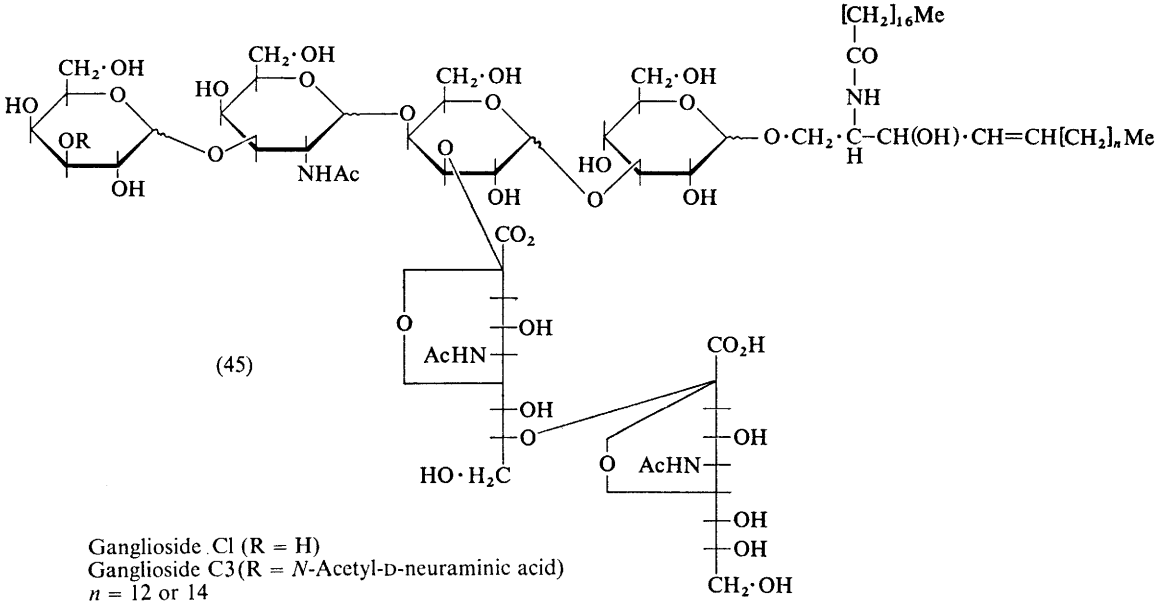
(44)

Glycosidic linkages probably α—

Phytanyl≡3′,7′,11′,15′-tetramethylhexadecyl

The structures have been reported[417] [see (45)] of four hexosamine-containing gangliosides (C1—C4) from human brain, based on acid hydrolysis, methylation, and periodate oxidation studies. On treatment with neuraminidase they were converted into ganglioside A2[418] with the concomitant release of 1 mol. of *N*-acetyl-D-neuraminic acid from ganglioside C1, 2 mol. from each of gangliosides C2 and C3, and 3 mol. from ganglioside C4. A dimeric form of *N*-acetyl-D-neuraminic acid could be prepared in small amounts from ganglioside C1. C2 was an isomer of C3, and differed from it in that one of the two galactosyl residues of ganglioside C2 was susceptible to oxidation by periodate. Ganglioside C4 resembled C3 but had a fourth neuraminyl residue at position 8 of one of the two

[413] M. Kates, B. Palameta, M. B. Perry, and G. A. Adams, *Biochim. Biophys. Acta*, 1967, **137**, 213.
[414] R. W. Walker and C. P. Bastl, *Carbohydrate Res.*, 1967, **4**, 49.
[415] D. E. Brundish, N. Shaw, and J. Baddiley, *Biochem. J.*, 1967, **105**, 885.
[416] M. G. Rumsby, *J. Neurochem.*, 1967, **14**, 733.
[417] E. Klenk, L. Hof, and L. Georgias, *Z. physiol. Chem.*, 1967, **348**, 149.
[418] E. Klenk and L. Georgias, *Z. physiol. Chem.*, 1967, **348**, 1261.



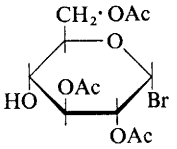
(45)

Ganglioside Cl (R = H)
Ganglioside C3 (R = *N*-Acetyl-D-neuraminic acid)
n = 12 or 14

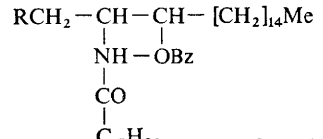
terminal D-neuraminyl residues of ganglioside C3. C1 and C3 were the two main components of ganglioside fraction C.

Rat brain contained enzymes which catalysed the hydrolysis of monogangliosides to their component sugars, fatty acid, and sphingosine.[419] An enzyme that catalysed specifically the cleavage of the terminal D-galactosyl residue in D-galactosyl-D-galactosyl-D-glucosyl ceramide to galactose and lactosyl ceramide was present in various tissues from rat and also in human small intestine.[420]

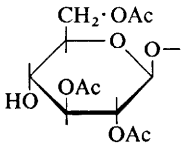
Syntheses have been reported[421] of 1-*O*-(4-*O*-β-D-hexopyranosyl-β-D-glucopyranosyl)dihydroceramides. For example, condensation of 2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (46) with 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine (47) gave the dihydrocerebroside



(46)



(47) R=OH

R= 

(48)

derivative 3-*O*-benzoyl-*N*-octadecanoyl 1-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-DL-dihydrosphingosine (48) which, on reaction with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-(or D-galacto-)pyranosyl bromide, gave dihydroceramide dihexosides that were β-D-(1 → 4)-linked.

[419] S. Gatt, *Biochim. Biophys. Acta*, 1967, **137**, 192.
[420] R. O. Brady, A. E. Gal, R. M. Bradley, and E. Mortensson, *J. Biol. Chem.*, 1967, **242**, 1021.
[421] H. M. Flowers, *Carbohydrate Res.*, 1967, **4**, 42.

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